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ABSTRACT BOOK

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001. Barry Firkin Oration: Well within 6 degrees of separation: From ristocetin to collagen binding and beyond.

Favaloro E

NSW Health Pathology, Westmead Hospital

Barry Firkin was an esteemed and respected scientist in the field of haematology with a broad clinical and academic interest. One of his most marked achievements, however, was the discovery that ristocetin promoted platelet aggregation in a von Willebrand factor (VWF) dependent manner, paving the way for the development of several diagnostic assays based on this finding. Undeniably, the ristocetin induced platelet aggregation (RIPA) and VWF ristocetin cofactor (VWF:RCo) assays are now part of the standard repertoire of laboratory tests for identification or exclusion of von Willebrand disease (VWD), in turn the most common bleeding disorder (arising from defects or deficiency of VWF). Indeed, VWF:RCo is still today considered the surrogate gold standard 'functional' or VWF 'activity' assay in VWD diagnostics. This presentation will explore VWD diagnostics, in part as a historical journey, and reflect on the many milestones and changes over recent decades. One milestone along this journey, for example, was the development of the VWF collagen binding (VWF:CB) assay as a second 'functional' or VWF activity assay in VWD diagnostics. Rather than being a replacement for VWF:RCo, the VWF:CB is seen as a supplementary assay that reflects a surrogate of one function of VWF, namely subendothelial matrix adhesion, as part of the process of attaching platelets to damaged tissue, and subsequent thrombus formation. The VWF:RCo, in turn, reflecting a surrogate of a complementary function of VWF, being platelet adhesion, as part of the process of facilitating platelets to attach to each other and to damaged tissue, and thus also aiding subsequent thrombus formation. More recently, this functional surrogate of VWF binding to platelets, aka the classical VWF:RCo assay, has been morphed into a variety of 'glycoprotein Ib (GPIb) – binding assays', and has even spawned a new ISTH SSC recommended nomenclature, including terms such as VWF:GPIbR and VWF:GPIbM. These are assays that may or may not use ristocetin, and generally do not even use platelets.

002. Ruth Sanger Oration: Reflections of a Journeyman Transfusionist

Flanagan P

New Zealand Blood Service

In May 1975 the World Health Assembly endorsed a resolution (WHA 28.72) urging member states to promote the development of national blood services based on the principle of voluntary non remunerated donation and in doing so requested further study into ethics and safety of commercial plasmapheresis. 35 years later a further resolution was endorsed (WHA 63.12) requesting member states to take all necessary steps to establish, implement and support the development of nationally co-ordinated BTSs with the aim of achieving self-sufficiency. During the intervening period dependency on plasma products derived from paid donor plasma increased significantly. Indeed in 2016 over 60% of the plasma products used globally were derived from this source raising questions as to whether plasma should now be considered as a strategic resource. This presentation will review the principles underpinning the concepts of voluntary non remunerated donation and self-sufficiency and in doing so consider whether they continue to be relevant in the 21st century.

003. Carl de Gruchy Oration: Chemotherapy, radiotherapy, immunotherapy, cellular therapy: the shifting sands of stem cell transplantation

Gottlieb D

University of Sydney

The last decade has seen an explosion in interest in the role of the immune system in curing cancer. For most physicians, immunotherapy has meant using monoclonal antibodies targeting antigens on the surface of cancer cells or more recently targeting inhibitory proteins on lymphocytes.

More recently, the use of immune system cells as therapies themselves, principally in the form of genetically modified T-cells bearing artificial antigen receptors, has garnered intense attention. T-cell therapy has a longer history than is generally recognised. It is inherently bound to allogeneic stem cell transplantation, a procedure that exerts the majority of its therapeutic (and much of its negative iatrogenic) effect through T-cell activity. The role of T-cells in allogeneic transplantation became apparent well after the birth of transplantation itself and the potential therapeutic effects of T-cells were recognised even later.

Stem cell donor-derived lymphocytes, first unmanipulated, then as purified antigen-specific cells were identified initially for their value as therapies in patients with refractory opportunistic infection. The herpes viruses EBV and CMV were the initial targets, but a gradual awareness that specific T-cells may play a role in a wide range of viral and fungal infections has emerged. A major inhibition has been the need to generate a specific T-cell product for each recipient, rendering T-cell therapy complex and expensive. The recognition that partially HLA matched 3rd party cryopreserved antigen-specific T-cells also have beneficial therapeutic effects largely circumvents this problem.

With the advent of T-cell therapies utilising artificial chimeric antigen receptors directed towards CD19, a new era was born. The recent FDA approval of autologous CD19 CAR T-cells for younger patients with refractory acute lymphoblastic leukaemia (albeit at a phenomenal price) has thrust T-cell therapy into the spotlight. CAR T-cells are being developed for a range of other indications.

Within the context of allogeneic stem cell transplantation, integration of specific T-cells directed at both infection and malignancy has the potential to be combined with removal of non-specific or alloreactive T-cells making a path towards a GVHD free, and infection and relapse reduced outcome clearer. Better specificity means better transplants. Less chemotherapy and radiotherapy, more immunotherapy and cellular therapy seems the way of the future.

004. What a headache, paediatric experience with Intragam 10.

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In March 2017, domestically supplied Intravenous Immunoglobulin (IVIg) Intragam P (CSL Behring) was replaced with Intragam 10 (CSL Behring). Intragam 10 is a more concentrated immunoglobulin product (10% versus 6%) and uses a different stabilising agent. Intragam 10 was introduced on the basis of two small cohort studies performed in adults, with no evidence to support its safety and efficacy in paediatric patients.

Aim

To review the safety profile and any adverse events associated with Intragam 10 at the Royal Children's Hospital, Melbourne.

Methods

A retrospective study of all Intragam 10 infusions from March to June 2017. Data collected included: demographics, dose, patient weight, indication, rates of infusion, patient vital signs, clinical assessments, re-presentation to hospital or correspondence with clinicians. Phone review was performed in the first month to determine if any late side effects post discharge.

Results

212 Intragam 10 infusions given to 108 patients were analysed. Median age 9.6 years, range 0 – 19.8 years. Median dose 0.8g/kg; range 0.2g/kg to 2.1g/kg.

Thirty five percent (76/212) Intragam 10 infusions were associated with adverse side effects or altered vital signs. Most commonly headaches were reported, 13/212 (6%) infusions. One instance of suspected clinical aseptic meningitis, 1/212 (0.5%).

Hypertension and bradycardia were commonly noted during infusions, hypertension alone 15/212 (7%), bradycardia alone (4.7%) and bradycardia with hypertension 6/212 (3%) of infusions.

Four cases of mild allergic reactions (1.9% infusions) and three episodes of anaphylaxis (1.4% infusions).

Two cases of intracranial haemorrhage within 96 hours of IVIg infusion were noted in the review.

Conclusion

Our study provides evidence that data from small studies in adults shouldn't be extrapolated to children. Paediatric recipients have high rates of adverse reactions and abnormal vital signs to Intragam 10. No national haemovigilance system exists to capture adverse events to IVIg and this deserves attention.

005. Proposed new low-frequency antigen in the Augustine system associated with hemolytic disease of the newborn

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Background

An investigation of a male infant with a severe case of haemolytic disease of the fetus and newborn revealed a maternal antibody reactive with paternal and cord red cells (RBC) but non-reactive against all panel cells tested. The maternal antibody also reacted against RBC from an older sibling and 3 members of the extended paternal family. As no known RBC variant was identified in these family members specimens were submitted for investigation for novel genetic variants.

Brief methods

Maternal, paternal and cord blood samples were provided along with samples from the older sibling, 2 paternal aunts and paternal grandmother. Targeted blood group exon and flanking intron sequencing for 33 blood group loci was performed using the TruSight™ One Sequencing Panel. CLC Genomics Workbench was utilised to call variants.

Results

For infant, older sibling, father, 2 paternal aunts and paternal grandmother, who were all serologically positive for the RBC antigen, analysis revealed a novel variant, c1159A>C, in the SLC29A1 gene (Genbank Number MF034879). The mutation was identified reliably, it was found in 56 of 116 DNA sequence reads in the paternal sample and 30 of 51 DNA sequence reads of the cord blood sample, consistent with heterozygosity. A similar pattern consistent with heterozygous inheritance was noted in all cases. This variant was not detected in the maternal sample.

Discussion

The nucleotide substitution c1159A>C is a missense mutation (p.Thr387Pro) in the SLC29A1 gene encoding the ENT1 protein. The loss of the AUG1 antigen of the Augustine blood group system is associated with a p.Glu391Lys (located in the 5th extracellular loop of ENT1) four residues from the p.Thr387Pro novel variant reported here. HDFN has been reported in pregnancy in an AUG1 negative mother. These data are consistent with antibodies to a proposed new antigen in the Augustine blood group system causing severe HDFN.

006. 50 years of RhD immunoglobulin (Anti-D) therapy in Australia - a public health success story

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Aim

We review the outcomes of Australia's RhD immunoglobulin (Ig) program, which celebrates its 50th year. This plasma-derived product protects RhD negative women from forming antibodies that cause haemolytic disease of the fetus and newborn (HDFN) in subsequent pregnancies. Issues of maintaining self-sufficiency of supply are outlined.

Method

The RhD Ig program began in 1967. Plasma was collected from donors with preformed anti D, or donors deliberately immunised to form anti-D. In 1969, Australia became the first country to provide RhD Ig, free of charge, to every pregnant RhD negative woman in need. A 250IU 'minidose' was introduced in 2001 and staged implementation of routine antenatal prophylaxis began in 2002, being completed in 2006.

Results

The program has maintained self-sufficiency for most of its history. More than 3 million doses of RhD Ig have been issued. Records for Victoria show that HDFN deaths have declined from 1.1 per thousand total births in 1967 to an average of 0.01 per thousand since 2000. Many thousands of babies have also been protected from non-fatal, but debilitating lifelong effects of HDFN.

Production of RhD Ig currently relies upon the plasma donations of only about 130 donors. Many are reaching retirement age and others may become ineligible to donate due to unrelated medical conditions. Recruitment of new donors presents an ongoing challenge. DNA testing to determine the RhD type of an unborn baby has been developed and has the potential to be used in future to ensure that anti-D is only given in pregnancies with an RhD positive fetus. However, as no viable alternative for RhD Ig is available, our need for donors continues.

Conclusion

Australia's RhD Ig program is a great tribute to its dedicated donors: HDFN is now a rare disease. However the challenges in maintaining supply will be ongoing.

007. TRUSTT me – transfusing RBCs in thalassaemia major, it's not cheap! TRUSTT study initial results.

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Background

No Australian data exist on costs of red blood cell (RBC) support for transfusion-dependent thalassaemia patients. This information is needed to inform management decisions, including understanding cost-effectiveness of new treatments. A previous European and US study (Shander et al, Transfusion 2010) in non-transfusion-dependent patients reported RBC product costs accounting for up to 32% of total transfusion costs.

Aim

Determine the true cost of RBC transfusion for transfusion-dependent thalassaemia in Australia (TRUSTT Study).

Method

A time-driven, activity-based, bottom-up costing of clinical, laboratory and administrative processes for outpatient and inpatient RBC transfusions for transfusion-dependent adult thalassaemia patients at Monash Medical Centre (MMC) was performed.

Detailed process maps with timings were developed for every procedure undertaken during March 2017. Direct and indirect costs (including personnel, consumables, equipment, clinical and testing procedures) were calculated, including costs of managing long-term consequences of transfusion, and other complications. Expert opinion was obtained where processes were unable to be timed.

Results

During the study period, 15,463 RBCs were issued in Victoria, of which MMC received 1,443 (9.3%). Of these, 478 (33%) were transfused to 117 adult thalassaemia patients, accounting for 3.1% of all RBCs issued in Victoria.

Thirty-two processes were mapped including prescription, sample collection, laboratory activities including inventory management, administration and follow-up of RBC transfusion.

Complexity of RBC requirements is demonstrated by 53% patients having a historically positive antibody screen, and 27.4% requiring antigen negative RBCs, although only 6.8% currently have a positive antibody screen.

For iron chelation, 79% patients received deferasirox, 18% patients received desferrioxamine, 3% received both.

Formal costing analysis is underway.

Conclusion

This study provides new data on the complexities of transfusion and other (e.g. chelation) support for an important group of transfusion-dependent patients. Detailed cost data determined as part of this study will be valuable for clinicians, hospital management, governments, blood services, patients and the broader community.

008. Propensity for alloantibody formation in transfusion-dependent patients

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A collection of single nucleotide polymorphisms (SNPs) within immunological genes and signalling pathways derived from gene chip screening proved useful in predicting anti-D immunoglobulin production for RhD-immunised healthy blood donors (Tan et al., 2015). It is uncertain whether these same SNPs could have predictive value for patients that may receive antigen-incompatible blood transfusion. We hypothesise that these identified genetic factors could be useful for predicting alloantibody formation in transfusion-dependent patients. Opportunities to conduct such studies are limited; patients are not deliberately transfused with non-self antigens. However, in the course of transfusion support to patients requiring red cells, patients may receive phenotype mismatched units. Regular transfusion patients (n=47) at a Sydney metropolitan hospital were assigned a Responder or a Non-Responder profile based on their alloantibody/autoantibody status. Older Thalassaemia patients were more likely to have developed antibodies than their younger counterparts (p value = 0.033). DNA was extracted from Thalassaemia patients (n=42) and genotyped for target SNPs and their predicted Responder profile generated using our predictive model. Responder Thalassaemia patients were significantly associated with 3 SNPs (TSLP, p value = 0.002; IGF1R, p value = 0.033; BLNK, p value = 0.007). The predictive model predicted 15 Thalassaemia patients currently assigned as a Non-Responder based on their alloantibody/autoantibody status as likely to be Responders. This could indicate that these 15 Thalassaemia patients have a higher propensity to develop alloantibodies, and so should continue to receive fully matched phenotyped red blood cell transfusions. Longitudinal follow up of these patients may determine if the predictive model was accurate.

Tan, J.C.G., Armstrong, N.J., Yuan, F.F., Flower, R.L., Dyer, W.B., 2015. Identification of genetic polymorphisms that predict responder/non-responder profiles to the RhD antigen. *Molecular Immunology* 68, 628-633.

009. Dual BH3-mimetic targeting of BCL-2 and MCL1 is efficacious and well-tolerated in Acute Myeloid Leukemia

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Aim

To determine the efficacy of targeting pro-survival proteins BCL-2 and MCL1 in pre-clinical models of AML.

Background

Identification of a chemotherapy-free option for AML represents a highly desired research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics able to inhibit pro-survival BCL-2 (Roberts *et al*, NEJM 2016), BCL-X_L (Lessene *et al*, Nat Chem Biol, 2013) and MCL1 (Kotschy *et al*, Nature 2016). We hypothesize that simultaneous pharmacological targeting of BCL-2 and MCL1 will enhance apoptosis of AML blasts, without increased toxicity to non-malignant cells.

Methods

S55746 (BCL-2 inhibitor) and S63845 (MCL1 inhibitor) were obtained from Servier and A1155463 (BCL-X_L inhibitor) from WEHI. Primary AML cells were obtained from patients providing informed consent. For *in vivo* experiments, NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) or NOD/Rag^{-/-}/Il2rg^{tm1Wjl} (NRGS) mice were used.

Results

S55746 and S63845 showed strong synergy in primary AML patient samples tested, suggesting that a dual BH3-mimetic targeting approach was highly efficacious. Remarkable anti-leukemic activity across a spectrum of AML cases with diverse cytogenetic and molecular pathologies was observed. Bioluminescent imaging of NSG mice showed rapid and sustained clearance of xenografted MV4;11 or OCI-AML3 cells, translating into significant prolongation of survival from combined S55746 + S63845. Patient-derived xenograft models revealed rapid reduction of established AML in the bone marrow after one week of treatment. Tolerability of this approach was confirmed in normal CD34+ stem and progenitor cells in short-term cell culture and long-term (2-3 weeks) clonogenic assays and from histological and biochemical examination of mice treated for up to 8 weeks at doses shown to be highly efficacious against AML.

Conclusions

Dual BH3-mimetic targeting of BCL-2/MCL1 induces synergistic cytoreduction of human AML cell line and primary AML samples *in vitro* and *in vivo*. We therefore report for the first time, that dual pharmacological inhibition of BCL-2/MCL1 represents a novel approach to treating AML and with an acceptable therapeutic safety margin. Our results support the translational investigation of dual BH3-mimetic targeting in the clinic.

010. Circulating tumour DNA genomic characterisation identifies early molecular lesions and clonal architecture in lymphoid malignancy

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Background and aims

DNA shed from tumour cells (known as “circulating tumour DNA” or ctDNA) can be detected in the peripheral blood of patients with haematological malignancy offering a less invasive approach to obtaining tissue for genomic characterisation. We aimed to perform comprehensive genomic characterisation from ctDNA in a diverse range of lymphoid malignancy.

Methods

Libraries were made from ctDNA collected from patients with a diverse range of lymphoid malignancies. Libraries were captured using the Peter MacCallum Cancer Centre (PMCC) PanHaem hybridisation-based next generation sequencing panel and sequenced on an Illumina NextSeq. The PMCC PanHaem panel targets 313 genes recurrently mutated in haematological malignancy as well as the immunoglobulin heavy chain locus.

Results

ctDNA from patients with a range of lymphoid malignancies (including diffuse large B-cell lymphoma, Burkitt lymphoma, angioimmunoblastic T-cell lymphoma and myeloma) was assessed. Comprehensive genomic characterisation including detection of sequence variants, genome-wide copy number changes and immunoglobulin heavy chain translocations was possible from ctDNA. Significant differences were observed in the relative allelic burdens of sequence variants, copy number changes and translocations between the cellular tumour and the ctDNA compartments. Molecular lesions that are classically considered to be late clonal events (e.g. RAS mutations in myeloma) were present at relatively low allelic burden in the ctDNA when compared to early “establishing” genomic lesions (e.g. t(11;14)). A marked difference in the spectrum from aberrant somatic hypermutation (aSHM) associated mutations was noted between the cellular DNA and ctDNA compartments consistent with the phenomenon of aSHM being an ongoing process in lymphoid malignancy.

Conclusions

Comprehensive genomic characterisation of lymphoid malignancy is possible from ctDNA and is of particular utility for detecting early clonal events. This technique is therefore of significant clinical value given the clinical relevance of these lesions in lymphoid malignancies.

011. Mass cytometry reveals CMV effect on immune recovery in HSCT patients undergoing adoptive T-cell therapy

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Aim

Adoptive cell therapy (ACT) with virus-specific T-cells (VST) has been shown to be safe and effective in controlling viraemia in recipients of haemopoietic stem cell transplant (HSCT). The fate of transferred cells is not well understood. We sought to investigate this with immune profiling by mass cytometry.

Methods

Peripheral blood samples were assessed with a 37 marker panel of metal-labelled antibodies acquired on the CyTOF2 mass cytometer to identify 75 canonical immune subsets per sample. Data was analysed with unsupervised and supervised clustering algorithms to identify immune signatures. Significance of microarray was used to identify the cell subsets that distinguished the clusters. t-stochastic neighbour embedding (tSNE) was used to correlate immune signatures with clinical variables.

Results

Samples from 10 healthy donors and 27 HSCT recipients who had received ACT in clinical trials were assessed. ViSNE revealed highly detailed relationships of the 75 cell subsets within individuals and between patients longitudinally. Unsupervised clustering revealed three major immunological signatures. Healthy individuals were clearly distinguished from all patients and no patient developed a normal immune profile. In a subset of HSCT samples a distinct immune phenotype characterised by increased CD8, CD4, B cells, NK cells, NKT cells, plasma cells and plasmacytoid dendritic cells could be identified. This signature was most strongly correlated with CMV reactivation and absence of GVHD.

Conclusion

Mass cytometry reveals an unprecedented level of detail regarding the relationships of immune subsets to one another and their changes over time. It is able to identify distinct global immune signatures in patients undergoing HSCT and ACT. We have identified an immune signature associated with CMV reactivation that may indicate a broader effect of CMV on immune recovery than previously recognised. Further studies are required to define the contribution of clinical parameters, including ACT, to these immune signatures.

012. Longer follow-up of the response-adjusted therapy for advanced Hodgkin lymphoma (RATHL) trial: longer follow up confirms efficacy of de-escalation after a negative interim PET scan (CRUK/07/033, ALLG HD8).

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Aim: This RCT tested whether PET-CT after 2 months of ABVD could guide subsequent treatment for patients with advanced I Hodgkin lymphoma (HL).

Method: Adult patients with newly diagnosed HL (IIB–IV, or IIA with bulk or ≥ 3 sites) underwent interim PET-CT scans after 2 cycles of ABVD (PET2). Images were reviewed centrally using the 5-point scale as negative (1–3) or positive (4–5). Pts with negative scans were randomised to ABVD or AVD for 4 more cycles. Pts with positive scans proceeded to either BEACOPP-14 or EscBEACOPP.

Result: Of 1202 eligible pts, 952 were PET negative and eligible for randomisation to ABVD/AVD. With a median 52 months follow-up, 3yr PFS for ABVD was 85.4% (95% CI: 81.9 – 88.4), and AVD 84.0% (80.3-87.1). The 1.2% difference in 3yr PFS (95% CI -3.7 - 4.8) lies within the predefined non-inferiority margin of 5%. There was a similar 5yr PFS of 82.7% and 80.6% and OS of 95.3% (93.7 – 97.0) and 95.0% (92.1 – 96.8) for ABVD and AVD respectively. Among 172 pts with a positive PET2, 5yr PFS was 65.7% (57.9 – 72.5) and 5yr OS 85.1% (78.3-89.9%).

Of 197 pts with bulky stage II HL PET2 was negative in 147 (75%). 3yr PFS was 89% (82.5 – 93.0) with no significant difference between ABVD/AVD, RT/no-RT, presence/absence of a residual mass, or PET score (1-3). The remaining 39 pts with bulky stage II HL and a positive PET2 received BEACOPP. Of the 11 pts receiving RT, there was just 1 progression, despite only 5 reaching conventional CT based CR

Conclusion: The primary endpoint of the RATHL study has been met, excluding a 5% inferior 3yr PFS following de-escalation after a negative interim PET-CT. With the caveat of a non-randomised subgroup, in pts with bulky stage II, those who achieve a negative PET2 have excellent outcomes without radiotherapy.

Keywords: ABVD; Hodgkin lymphoma (HL); positron emission tomography (PET).

013. Identification of two oestrogen-sensitive microRNAs as direct inhibitors for tissue factor and factor VIII genes

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Aim

High oestrogen (E₂) levels are associated with a hypercoagulable state and increased risk for venous thromboembolism, but the underlying mechanisms are not defined. We cultured HuH-7 liver carcinoma cells in the absence and presence of E₂, and identified multiple E₂-sensitive microRNAs (miRNAs) via NanoString nCounter[®] miRNA array, of which eight candidate miRNAs were predicted to target tissue factor (*F3*) and/or factor VIII (*F8*) genes. Therefore, the aim of this study was to investigate the direct inhibitory effects of the E₂-sensitive miRNA candidates on tissue factor and/or factor VIII gene expression.

Method

Full length-*F3* and *F8*-3'UTR sequences were cloned into the pRR5DUO dual luciferase reporter vector, co-transfected with 50nM miRNA precursors (negative control or candidate miRNAs) in HuH-7 cells, then assayed for Gaussia and firefly luciferase activities, at 24h post-transfection. To confirm direct miRNA-mRNA interactions, site-directed mutagenesis was employed to remove the putative miRNA seed sequences from the pRR5DUO-3'UTRs and assayed for miRNA-dependent inhibition of luciferase activity. Student's t-test was used to determine the statistical significance.

Result

The *F3*-3'UTR contained putative binding sites for six miRNA candidates, but only miR-365a-3p was shown to significantly inhibit *F3*-3'UTR-dependent luciferase activity (p<0.05). The inhibitory effect was abolished when the miR-365a-3p seed binding site was removed. Five candidate miRNAs were predicted to bind in this *F8*-3'UTR, of which only miR-548aa significantly inhibited *F8*-3'UTR-dependent luciferase activity (p<0.05). Deletion of the miR-548aa binding site on *F8*-3'UTR completely removed the inhibitory effects of miR-548aa on *F8*-3'UTR-dependent luciferase activity.

Conclusion

Two E₂-responsive miRNAs, miR-365a-3p and miR-548aa, were identified as novel regulators of *F3* and *F8* expression, respectively. Ongoing work is to characterise miR-365a-3p and miR-548aa regulation on *F3* and *F8* protein expression and function. This elucidates the E₂-miRNA regulation network contributing to E₂-associated thrombotic risk, which may be useful biomarkers for thrombosis or the development of miRNA-based therapies.

014. Demonstrating GPIIb α -thrombin interaction in procoagulant platelet formation leads to identification of potential specific inhibitors

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Procoagulant platelets are a highly activated platelets-subpopulation which support thrombin generation. We previously reported that coronary artery disease (CAD) patients have excess procoagulant platelet response to thrombin. Thrombin directly activates platelets via cleavage of PAR1 and PAR4, but is also known to interact with GPIIb α via exosite II.

Aim: To understand the mechanism of thrombin induced procoagulant platelets and to generate novel antiplatelet therapies.

Hypothesis: Involvement of both PAR-dependent and GPIIb α -thrombin interaction pathways.

Methods: Procoagulant platelets detected by FACS (GSAO, P-selectin, lactadherin) in whole blood and washed platelets after treatment with PAR, GPIIb α , exosite II inhibitors and heparins prior to thrombin stimulation.

Results: Preincubation with combined PAR1 and PAR4 inhibitors reduced procoagulant platelet formation by 40 \pm 5.7%, indicating PAR pathways are not sufficient for thrombin effect. Thrombin exosite II was implicated by reduction of thrombin induced procoagulant platelets by exosite II targeting aptamer (92 \pm 4.1%) and heparin. Interaction with GPIIb α was demonstrated using competition studies with recombinant soluble GPIIb α , glyocalicin (49 \pm 7.7%). Involvement of the N-terminus of GPIIb α was implied by use of NK protease which cleaves GPIIb α proximal to the thrombin binding site (39.5 \pm 2.9%). Additive inhibition of procoagulant platelet formation after thrombin stimulation was achieved by combination of GPIIb α cleavage and PAR1 and PAR4 inhibition. Having demonstrated a role for displacement of thrombin from GPIIb α in targeting procoagulant platelet formation, we evaluated the effect of potential inhibitors of this binding on procoagulant platelet formation in healthy controls and CAD patients and one (inhibitor X) resulted in selective targeting of procoagulant platelets (40.5 \pm 10% and 40.6 \pm 9.5% respectively).

Conclusion: The GPIIb α /thrombin exosite II interaction is strongly implicated in thrombin induced procoagulant platelet formation. We identify a potential therapeutic that specifically targets procoagulant platelets as a possible adjunct anti-platelet therapy in CAD. We aim to confirm our results *ex vivo* and *in vivo* by using PAR4 knockout and GPIIb mutated mice.

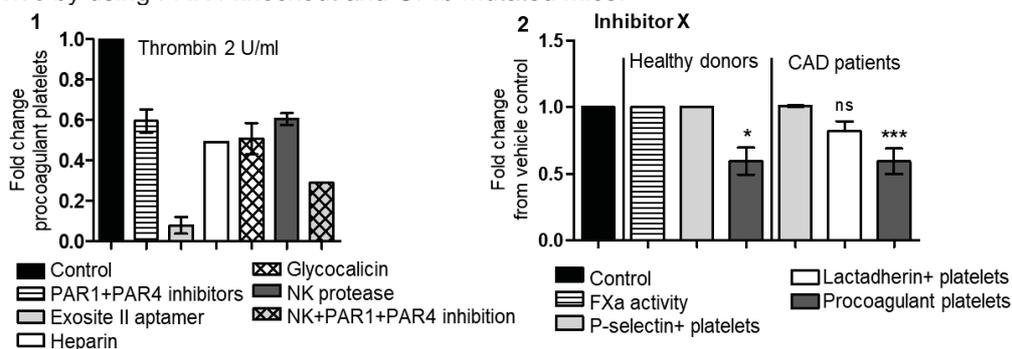


Figure 1: Whole blood was treated with PAR1 and PAR4 inhibitors or exosite II aptamer and washed platelets were treated with heparin, glyocalicin, NK protease or NK and PAR inhibitors prior to thrombin stimulation (2 U/mL). **Figure 2:** Whole blood from healthy donors (n=4) or CAD patients (n=10) was preincubated with control or inhibitor X (73 ug/ml) prior to thrombin stimulation (2 U/mL). Data are expressed as fold change from baseline (*p<0.05, ***p<0.001).

015. Haematopoiesis aging and the molecular basis of myeloid neoplasms/MDS - clinical implications

Malcovati L

University of Pavia

Recently, large studies provided consistent evidence of age-related hematopoietic clones, driven by mutations of genes recurrently mutated in myeloid neoplasms, and associated with increase in the risk of hematologic cancer. Mutations were found in up to 10% of persons 70 to 79 years of age, and 20% of persons 90 years of age or older. Clonal expansions most frequently involved somatic mutations in three genes implicated in epigenetic regulation, *DNMT3A*, *TET2*, and *ASXL1*. The vast majority of subjects carrying detectable mutations had only one mutation in the set of examined genes, supporting the hypothesis that these persons had clones harboring only an initiating lesion.^{1,2} The available studies suggests that genes involved in DNA methylation or chromatin modification are initiating lesions driving the expansion of a premalignant clone. The occurrence of additional cooperating mutations is then resulting in the transformation of the hematopoietic cell. Conversely, experimental and clinical data suggest that mutations in *SF3B1* may be the initiating and the only mutation sufficient to promote myelodysplasia.³

The available evidence clearly indicates that greater understanding of the molecular basis may improve our management of myeloid neoplasms, having the potential to impact on the diagnosis, classification, prognostic assessment and patient allocation to the appropriate health care intervention, as well as to drive the development of novel targeted treatment modalities. Recent data suggest that mutation analysis may be a valuable complement to the current diagnostic workup of suspected myeloid neoplasm, having a high predictive value for identifying individuals with, or at high risk of developing a myeloid malignancy.⁴ In addition, the identification of specific associations between genotype and disease phenotype is the basis for recognizing disease entities according to distinctive genetic profiles. Recently, *SF3B1* mutation was shown to identify a distinct entity within myelodysplastic syndromes (MDS).⁵ Interestingly, MDS patients with *SF3B1* mutation showed a high response rate to targeting aberrant TGF- β signalling implicated in ineffective erythropoiesis, and pre-clinical studies provided evidence that cells carrying a mutated splicing factor are selectively vulnerable to therapeutic splicing modulation.⁶

Mutations in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* were identified as independent predictors of poor overall survival in patients with MDS, providing the proof of concept that the combination of somatic mutations with conventional risk factors may improve prognostic stratification in patients with MDS.⁷ More recently, mutations in *RUNX1*, *NRAS*, *SETBP1*, and *ASXL1* were independently associated with survival in chronic myelomonocytic leukemia, and were integrated into a clinical/molecular prognostic scoring system.⁸ Moreover, recent studies showed that mutation profile may also provide useful information to predict response to specific treatment modalities. In fact, *TET2* mutations were found to predict response to azacitidine or decitabine,⁹ and mutations in *TP53* and RAS pathway were associated with decreased survival after allogeneic stem cell transplantation.¹⁰

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016. Generation of blood cells from pluripotent stem cells for clinical use

Ghevaert C

Cambridge Institute for Medical Research

Platelet transfusions to thrombocytopenic patients are increasing by 7-10% per year. We are currently entirely reliant on donor-derived platelets, which have limitations: short shelf-life and precarious supply chain, risk of donor-derived transmitted infections and issues of HLA mismatch in chronic recipients. We are aiming to develop protocols to produce platelets in vitro from a renewable source of stem cells - human pluripotent stem cells (hPSCs) - using a methodology and reagents compatible with the production of a clinical grade commercially viable product.

First we developed a chemically defined forward programming (FoP) approach to produce megakaryocytes (MKs) from hPSCs based on the overexpression of 3 key transcription factors (TFs; GATA1, FLI1 and TAL1) driven by lentiviral vectors. This FoP protocol generates pure MK cultures (>80% CD41+ CD42+ cells) which expanded in vitro for several months culminating on average to 2x10⁵ MKs per starting hPSC with minimum cytokine requirement and cell handling. Second, we addressed the challenge of the low platelet number produced per MKs in vitro (at best 10 platelets per MK whilst in vivo it is estimated that MKs produce >1000 platelets per cell). To improve platelet release and harvest, we recreated the characteristics of the bone marrow vascular niche in vitro. In the first instance, we use collagen-based 3-dimensional porous scaffolds to recreate the physical 3-dimensional space. We then screened a library of 350 recombinant ectodomain proteins in order to identify cell-to-cell contact signal proteins that promote proplatelet formation. The proteins can be immobilised to collagen 3D-scaffolds leading to an increase in platelet yield of an order of magnitude.

Finally, we have focused our research in order to translate these discoveries to an economically viable, GMP compatible large-scale manufacturing process which includes the development of an inducible cassette to forward programme the iPSCs without the need of lentiviral vectors replacing R&D reagents with GMP equivalent and developing new culture media avoiding the use of expensive proprietary compounds. Screening 18 clinical grade embryonic stem cells and induced pluripotent stem cell lines to identify the best line in terms of MK production by FoP. Using genome editing to create HLA null "universal" platelets.

017. Evolution of treatment in TTP

Peyvandi F
University of Milan

Abstract not provided

018. Personalised treatment of MM

Jackson G

Freeman Road Hospital

It is said we are entering the era of personalised medicine for cancer patients and particularly for patients with Multiple myeloma. Therapeutically this may be true but clinically we have always practised personalised medicine. We do not apply a one size fits all approach.

Decisions we make include:

Do we treat or not to treat patients with smouldering myeloma?

How do we decide if someone is transplant eligible v transplant ineligible?

Frailty adjusted therapy?

Should we adjust therapy according to response or MRD status?

Do we adjust therapy according to cytogenetic risk?

How do we approach patients with significant renal impairment at presentation?

When to treat at relapse?

What treatment should we choose at relapse?

Are there novel biomarkers that point to the usefulness of novel therapeutic reagents?

This presentation will look at our decision making and how we are already personalising therapy and how we might personalise treatment in the future?

019. The human genome: Whole genome sequencing and RBC/HPA antigen prediction

Westhoff C

New York Blood Center

Typing for red cell and platelet antigens by targeting single nucleotide polymorphisms (SNPs) is now commonplace, but there are limitations. SNP approaches do not detect all silent (null) alleles, resolve gene rearrangements, or enable full coverage of the gene needed to reliably determine ABO and RhD without serologic confirmation. Next generation sequencing (NGS) methods are fast replacing Sanger sequencing, and NGS approaches targeting the whole genome (WGS), rather than targeting specific genes, are rapidly being applied in many areas of medicine. Examples include programs like MedSeq and TopMed in the U.S., the 100,000 genomes project in the UK, as well as whole genome sequencing of target populations including the Australian Rare Diseases and Melanoma populations. This data can also be used in transfusion medicine to determine red cell and platelet antigens for patients requiring transfusion. However, without computerized algorithms capable of robustly converting phenotypes directly from non-targeted NGS data, the translation of NGS data is laborious and time-intensive and can only be performed by a few subject matter experts. We developed a comprehensive database and automated algorithm capable of rapid determination of all known RBC and PLT antigens using computer BAM file inputs from WGS data and validated this approach in 100 patient samples tested by serology and SNP. Individuals with hematologic malignancies and chronic illness may soon routinely get whole genome sequencing for diagnosis or treatment. Automated typing algorithms have the potential to transform the way safe blood products are routinely provided to patients. This approach offers a comprehensive, accurate and cost-effective method to potentially improve transfusion safety by more precise antigen matching, and represents one of the first applications of using genomic information for routine clinical benefit.

020. Personalised Anticoagulation

Nandurkar H

The Alfred

Medicine has always been personalised as we incorporate genetic, behavioural and environmental factors that affect disease and drug response into individual patient management decisions. The use of INR for pharmacodynamic modification of anticoagulation intensity is a well-known example of personalising anticoagulation and has the advantage of bypassing genetic variations that influence vitamin K antagonists (VKA) pharmacokinetics. An advantage of direct acting oral anticoagulants (DOACs) over VKAs is the capacity for effective anticoagulation without the need for monitoring, i.e. shifting the focus from personalised care to a 'one size fits all' approach. Apart from extremes of age, weight and renal function, this shift away from individualisation has proven to be equal to, or better than VKAs in terms of efficacy and safety. Genetic variations are well known to influence the activity of antiplatelet drugs such as clopidogrel, prasugrel and ticagrelor. While these drugs are generally effective, there is reported wide inter-individual variation in response to these drugs, defined by either laboratory parameters (ie, ex vivo measures of platelet aggregation) or clinical response (cardiovascular endpoints). For example, genetic factors contribute to almost 70% of the variability of the clopidogrel response on ADP-stimulated platelet aggregation. Also, heritable factors in molecular pathways directly and indirectly related to cyclooxygenase-1 (COX-1) significantly contribute aspirin-dependent platelet responsiveness. There is now a large body of evidence to suggest that the *CYP2C19**2 variant significantly impacts clopidogrel pharmacokinetics and pharmacodynamics and algorithms to guide selection between clopidogrel, prasugrel and ticagrelor have been developed based on upfront analysis of *CYP2C19* polymorphisms. The challenge remains in cost effectiveness of testing, as the effect size of genetic variations is not large. In the area of cancer-associated thrombosis, there is enormous interest in oncogene and onco-microRNA regulation of the coagulome. There is data that microRNAs that drive coagulation factors levels may influence cancer-associated coagulation; for example, miR-19a and miR-520g suppress tissue factor expression and may be associated with lower tumour thrombogenicity. This talk reviews the data available for the genetic cause for inter-patient variability of antiplatelet and anticoagulant drugs, its real world clinical impact and discusses personalising anticoagulation.

024. Pathophysiology and progress in the management of chronic GVHD

Hill G

Royal Brisbane & Womens' Hospital

The last 5 years has seen an enormous expansion in both preclinical and clinical research into chronic graft-versus-host disease (cGVHD). For the first time, we now have a clear understanding of the complex cellular and cytokine network that drives the fibrosis characteristic of disease. cGVHD is mediated by naïve T cells differentiating within follicular helper T cell (Tfh) and Th17/Tc17 patterns, generating IL-21 and IL-17A to drive pathogenic germinal centre (GC) B cell reactions and monocyte-macrophage differentiation respectively. The pathogenesis of cGVHD includes thymic damage, impaired antigen presentation and a failure in IL-2-dependent Treg homeostasis. Pathogenic GC B cell and macrophage reactions culminate in antibody formation and TGFβ respectively leading to fibrosis. Up until this point, treatment options have been largely limited to high and prolonged doses of steroids. Our new understanding has permitted the design of rational targeted therapeutics and at least half a dozen of these agents have entered clinical trials including janus kinase inhibition, bruton's kinase inhibition, ROCK2 inhibition, Syk inhibition, IL-2 mimetics and proteasome inhibitors. New logical approaches to cytokine inhibition with candidate therapeutics are now also emerging focussing on IL-12, IL-17A, IL-21 and CSF-1. The future looks encouraging such that we may now soon be able to effectively prevent and treat this devastating disease.

025. Chronic GVHD Therapy: Current status and future perspectives

Koreth J

Dana-Farber Cancer Institute, Harvard Medical School

Despite improvement in allogeneic hematopoietic stem cell transplantation (HSCT) outcomes, chronic Graft-versus-host disease (GVHD) remains a major cause of post-transplantation morbidity and increased non-relapse mortality. Current chronic GVHD treatment typically utilizes corticosteroids and other broadly immunosuppressive agents, despite their limited efficacy and considerable long-term toxicity. Improved chronic GVHD therapy remains a major unmet need. Advances in our understanding of the complex immune pathology of chronic GVHD, and the availability of novel agents targeting immunologic mechanisms and pathways implicated in cGVHD offers the possibility of therapeutic breakthroughs. The talk will provide an overview of the 'state of the art' in chronic GVHD, with an emphasis on novel therapeutic modalities that can rationally target immune dysregulation underlying chronic GVHD, and that are supported by both preclinical data and the results of early phase clinical trials. Highlighted therapies include novel B- and T-cell targeted modalities.

026. Novel therapies or management of renal impairment in myeloma

Jackson G

Freeman Road Hospital

The UK MRC XI trial has involved over 4,000 transplant eligible and transplant non-eligible patients with newly diagnosed multiple myeloma treated across 106 centres in the UK. All patients were randomised between Cyclophosphamide/revlimid/dexamethasone (CRD) and cyclophosphamide/thalidomide/ dexamethasone (CTD) with the dose of dexamethasone being attenuated for the older frailer patient.

Results will be presented for PFS/OS for the initial CTD(a) v CRD(a) randomisations, on the patients who have no response to Imid induction, on the results of the second induction randomisation (velcade/thalidomide/dexamethasone) VTD v no intensification for poor responders and the results of the randomisation between Revlimid maintenance and no maintenance. The outcomes of the VTD and maintenance randomisations will be analysed according to MRD status and cytogenetic status.

The response rates to (Carfilizomib/cyclophosphamide/Revlimid/dexamethasone) CCRD from the myeloma 11+ trial will also be presented.

The results to date will also be discussed in the light of results from other studies in the front line setting.

027. Amyloid management, new drugs/therapies

Kwok F

Abstract not provided

028. TCR knockdown with shRNA as a foundation for a piggyBac transposase-generated CD19-specific CAR T-cell bank

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Aim

Autologous CD19-specific CAR T-cells have shown remarkable efficacy against B-cell malignancies, but manufacture of individualised products is expensive, delays patient treatment and is unfeasible in patients with insufficient healthy T-cells. We sought to evaluate TCR suppression by short hairpin RNA (shRNA) in low-cost *piggyBac*-generated CAR T-cells, in order to generate a ready-made allogeneic product devoid of alloreactivity.

Methods

PiggyBac transposon plasmids were generated for expression of CAR19-28z alone, or additionally with shRNA against either TCR α -chain or TCR β -chain (shRNA designed by Takara Bio Inc). T-cells isolated by immunomagnetic separation were electroporated with *piggyBac* transposon and transposase plasmids, and expanded over 22 days via CD19 stimulation with IL-15 support. TCR⁺ cells were then enriched by immunomagnetic CD3 depletion in CAR T-cell cultures that had been transfected with shRNA, to create a final product. Final products were further cultured to ensure stability of TCR downregulation.

Results

All CAR T-cell products showed vigorous expansion over the first 22 days (minimum 100-fold). CAR expression on T-cells in final products was robust with CAR19-28z alone or with additional TCR α -chain shRNA (mean 96% and 98%, respectively), but was significantly lower with additional TCR β -chain shRNA (mean 56%). The proportion of TCR⁺ CAR T-cells was significantly greater in TCR α -chain and TCR β -chain shRNA-containing final products (mean 74% and 71%, respectively) compared to CAR19-28z alone (mean 5%). TCR inhibition was stable over a further 42 days with TCR α -chain shRNA, but not TCR β -chain shRNA (mean proportion TCR⁺ CAR T-cells 71% vs 1%, respectively).

Conclusion

CD19-specific CAR T-cells with stably suppressed TCR were successfully generated by introducing genes for both CAR and shRNA against TCR α -chain into T-cells via single-step genetic modification with the *piggyBac* transposase system. This platform could form the foundation for a bank of inexpensive allogeneic HLA-matched CAR T-cells with low GVHD potential, suitable for rapid treatment of many recipients.

029. Ex vivo expanded PRAME- and WT1-specific T lymphocytes for treatment of acute myeloid leukaemia

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Introduction

Adoptive immunotherapy with *ex vivo* expanded donor-derived tumour-specific T-cells may improve disease control following allogeneic transplant. We previously demonstrated high expression of preferentially expressed antigen in melanoma (PRAME) and Wilms tumour gene 1 (WT1) in primary AML samples. Here we sought to develop GMP compliant manufacturing methods for WT1 and PRAME specific T-cells from normal donors for use in adoptive immunotherapy.

Method

Peripheral blood mononuclear cells (PBMCs) or haemopoietic progenitor cells (HPC) from healthy donors were pulsed with PRAME or WT1 overlapping peptides. After 16 hours, enrichment of activated cells was performed using immunomagnetic beads. Cells were co-cultured with irradiated activation marker-negative fraction and supplemented with IL-2, IL-7 and IL-15. Cultures were stimulated with irradiated antigen-pulsed autologous cells after 10 days and subsequently every 7 days. Multiparameter flow cytometry was performed to assess cellular phenotype and cytokine response following antigen exposure.

Results

WT1 cultures (n=7) had mean expansion of 24-fold at day 25. 5/7 cultures demonstrated WT1 specificity by IFN- γ production (Responders: mean 38% of CD8+ cells, range 6-61). At end of culture, the mean percentage of CD3+ cells was 98.3% with CD4:CD8 ratio of 1:3.9. Central and effector memory T cells were 2.3% and 62% respectively of T cells, with 25% of all T cells expressing exhaustion marker PD1.

PRAME stimulated cultures (n=7) had mean expansion of 241-fold at day 25. 6/7 cultures showed PRAME specificity by TNF production (Responders: mean 10% of CD4+ cells, range 4-14.) Mean CD3+ percentage was 95.7% with CD4:CD8 ratio of 3.4:1. Central and effector memory cells were 18.4% and 79% respectively, with 25% T cells expressing PD1.

Conclusion

We successfully enriched WT1- and PRAME-specific T-cells from healthy donor PBMC and HPC. We plan to incorporate these into a phase I trial in which we administer myeloid leukaemia and infection specific T-cells post-transplant.

030. Preclinical testing of novel therapies for Down Syndrome acute megakaryoblastic leukaemia

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Rationale

Children with Down Syndrome (DS) have a 500 fold increased risk for developing acute megakaryoblastic leukaemia (AMKL), indicating genetics changes associated with DS impact progression to DS-AMKL. In addition, DS-AMKL patients have increased severe therapy-related toxicities, preventing the use of higher chemotherapy doses, and poorer outcomes following relapse. Novel therapeutic strategies are therefore required. Of particular interest is the potential to repurpose drugs in advanced pre-clinical models and clinical trials, that target early genetic events driving progression to and maintenance of DS-AMKL. Candidate genetic events include trisomy of genes such as ERG and mutation GATA1s.

Aim

Assessment of targeted signal pathway inhibitors for potential treatment of DS-AMKL.

Methods

Numerous drugs targeting signalling pathways implicated in progression to DS-AMKL were selected. Two human drug testing models were established: immortalised cells from ERG-high and ERG-low GATA1s-positive DS-AMKL, and iPSC lines derived from DS and non-DS subjects. Effects of gene downregulation and signal pathway inhibition were assessed *in vitro* and *in vivo*. Statistical analysis included ordinary one-way analysis of variance, t-tests, and non-linear regression.

Results

Analysis of ERG expression level was used to stratify DS-AMKL cell lines, and was shown to be elevated in DS-derived iPSC throughout haematopoietic differentiation. DS-derived iPSC were also confirmed to recapitulate DS-associated haematopoiesis with the expected increased frequency of all colony forming unit types ($p < 0.005$). shRNA-based reduction in ERG expression resulted in reduced production of CD34+ haematopoietic progenitor cells from DS-iPSC ($p = 0.09$), and significantly sensitised high-ERG DS-AMKL to cytarabine (decreasing IC₅₀ from 0.6 μ M to 0.3 μ M, $p < 0.05$). Targeted inhibitor AMKL-004 differentially reduced ERG-high leukaemic cell survival *in vitro* (ERG-high IC₅₀ 0.7 μ M, ERG-low IC₅₀ 11.9 μ M, $p < 0.001$), and synergistically reduced engraftment *in vivo* when combined with cytarabine ($p < 0.03$).

Conclusion

Targeted inhibitor AMKL-004 has potential as a treatment for DS-AMKL, and could be readily repurposed for clinical use.

031. CD123 Chimeric Antigen Receptor T-cells in Chronic and Acute Myeloid Leukaemia: pre-clinical *in vitro* studies.

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Introduction: Treatment for relapsed/refractory acute myeloid leukaemia (AML) and blast-crisis chronic myeloid leukaemia (BC-CML) is suboptimal. We are developing Chimeric antigen receptor (CAR) T-cell immunotherapy targeting CD123, which is overexpressed in these leukaemias. We generated four third-generation CD123-CAR constructs to evaluate their *in vitro* functional properties.

Methods: CD123 expression of bone marrow from healthy donors (HD), AML and BC-CML at diagnosis were assessed by flow cytometry. CD123-CARs were constructed, modified (*Figure 1.*) and cloned into a third-generation lentiviral system for effective T-cell transduction. T-cells from cryopreserved HD peripheral blood mononuclear cells were activated with CD3/CD28 Dynabeads and rIL-2, and transduced (72h post-activation; MOI 40). CD123-CAR T-cells were isolated (BD Fusion) using biotinylated Protein-L, expanded for 14 days and subjected to downstream functional assessment including CD107a degranulation in increasing Effector (E; CAR+ T-cells): Target (T; CD123+ cell line, KG1a) ratios and T-cell proliferation quantified using the CellTrace Violet dye.

Results: CD123 expression on CD34+CD38- stem cells were increased in primary AML (n=32) (66.9% \square 22.7, p<0.02) and BC-CML (n=10) (21.7% \square 18.1) compared to HD (n=10) (3.3% \square 7.2). CD123 was also increased in AML (51.1% \square 22.0, p<0.02) and BC-CML (17.3% \square 13.2, p<0.05) compared to HD (1.2% \square 1.3) in CD34- mature cells. T-cell transduction of up to 25% was achieved, viability was maintained >90% and programmed death-1 expression (a marker of T-cell exhaustion) was not altered. CAR+ T-cells were predominantly CD4+ (67% \square 5.8, n=6) versus CD8+ (20.1% \square 5.5, n=6), and displayed a central (CD3+CD27+CD45RO+) or effector memory (CD3+CD27-CD45RO+) phenotype. Both CD4/CD8+ populations from each CAR demonstrated robust antigen-specific degranulation against KG1a cells but not CD123- SUPB15 cells (both n=3). Similarly, CD123-CAR T-cells also proliferated in response to KG1a cells.

Conclusion: All CD123 CARs demonstrate potent effector functions against CD123+ target cells. Following *in vitro* assessment against patient samples, the most promising CAR will be assessed in murine *in vivo* functional studies prior to clinical development.

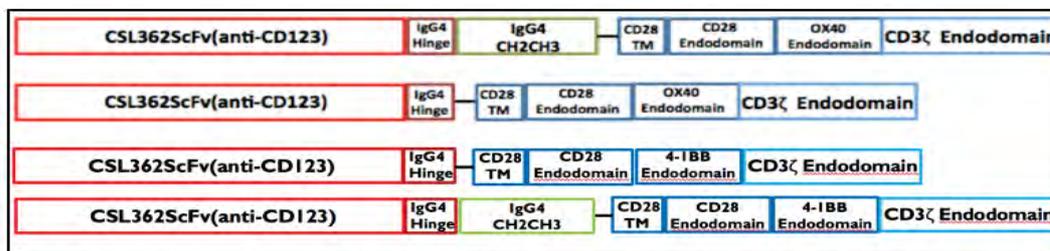


Figure 1. Schematic design of the CD123 3rd generation CARs. CSL362 ScFv was bound to co-stimulatory domains (i) CD28, 4-1BB and (ii) CD28, OX40 in tandem with CD3 chain with each connected to a CD28 transmembrane moiety containing either a (iii) long or (iv) short IgG4 hinge.

032. Dynamin inhibitors, a novel strategy to target multiple signalling pathways in leukaemia stem cells.

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Background

Early-thymocyte progenitor T-cell leukaemia (ETP-ALL) is a poor prognostic subtype of T-cell ALL in which mutations activating multiple cytokine signalling pathways is frequent. In particular, activating mutations of Notch and the IL-7-JAK-STAT pathway are observed in more than 50% of cases, allowing leukemic stem cells (LSCs) to survive in the absence of niche signals and high dose chemotherapy. Accordingly, Notch and JAK inhibitors have some therapeutic benefit but are limited by toxicity and ill-defined compensatory mechanisms.

Aim

To investigate the therapeutic potential of Dynole 34-2, an inhibitor of the GTPase Dynamin that is required for receptor-mediated endocytosis of multiple cytokine signalling pathways.

Method

Lmo2-transgenic (*Lmo2^{tg}*) mice were used as a model of human ETP-ALL. Eight week old mice were used to examine mechanism of action and efficacy on LSCs while syngeneic mice transplanted with *Lmo2*-derived ETP-ALL were used to test the therapeutic potential of Dynole 34-2 in combination with chemotherapy.

Results

In-vitro culture of LSCs with Dynole 34-2 showed increased apoptosis compared with immunophenotypically identical wildtype thymocytes. Flow-based assays confirmed inhibition of endocytosis and cytokine signalling (Notch and IL-7) by Dynole 34-2. Treatment of *Lmo2^{tg}* mice with Dynole 34-2 for 2 weeks selectively killed LSCs as demonstrated by a 100-fold reduction in LSC repopulating activity. This ability to target LSCs was enhanced when combined with multi-agent chemotherapy typically used for human ETP-ALL. Finally, the addition of Dynole 34-2 with chemotherapy was well tolerated and significantly prolonged survival including 25% long term cures.

Conclusion

These results demonstrate efficacy of inhibiting receptor-mediated endocytosis, a novel strategy to target multiple signalling pathways that maintain survival and chemo-resistance of LSCs.

No Conflict of Interest to Disclose.

033. Correlation between 10-colour flow cytometric minimal residual disease (MRD) and molecular MRD in adult ALL

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Background

MRD monitoring in ALL is a strong predictive factor and a stratification tool for treatment intensification. The currently accepted standard of molecular monitoring with either immunoglobulin heavy or kappa chain (IG) or T-cell receptor (TCR) quantitative PCR (qPCR) in Philadelphia negative (Ph-) ALL offers high sensitivity, but accessibility is limited by expertise, cost and turnaround time. Flow cytometric assays are increasingly utilised and improved sensitivity is seen with multi-parameter flow cytometry at 8 or more colours.

Aim

To compare 10-colour flow cytometry with molecular monitoring for detecting MRD in B-ALL.

Methods

We developed a 10-colour single tube flow cytometry assay (Figure 1). Samples were subject to bulk ammonium chloride lysis to maximise cell yields with a target of 1×10^6 events. Once normal maturation patterns were established, patient samples were analysed in parallel to standard molecular monitoring with either IG/TCR qPCR in Ph- disease or *BCR-ABL* qRT-PCR in Ph+ disease. Statistical analysis was performed in Graphpad Prism v7.0.

Results

Flow cytometry was performed on 47 samples from 16 patients. 13 samples were at diagnosis or morphologic relapse. An informative immunophenotype was identifiable in all patients; however a molecular assay could not be developed in one patient.

38 samples were tested for MRD by flow cytometry (Figure 2). In 2 samples, flow cytometric MRD was detected despite blinatumomab (anti-CD19) therapy.

27 samples were tested concurrently for MRD by both molecular and flow cytometric methods (Figure 3A and 3B). There was a strong correlation between molecular and flow cytometric MRD ($R^2=0.909$, $p<0.001$; Figure 3B). Correlation was strong with both IG/TCR-based ($n=16$; $R^2=0.955$, $p<0.001$) and *BCR-ABL*-based ($n=11$; $R^2=0.957$, $p<0.001$) assays. The cost was significantly lower than IG/TCR qPCR (eg. Cost for four time-points per patient approximately \$1200 vs \$3700).

Conclusion

Our 10-colour flow cytometric MRD assay attained sensitivity of $\leq 0.01\%$ in 87% of samples, and correlated strongly with molecular assays. This technique offers rapid and affordable testing in B-ALL patients, including cases where a suitable molecular assay cannot be developed.

034. Haploidentical stem cell transplantation is a suitable platform for patients lacking a HLA matched donor.

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Aim

Update outcomes of haploidentical (haplo) haematopoietic allogeneic stem cell transplantation (HSCT) at Westmead Hospital.

Methods

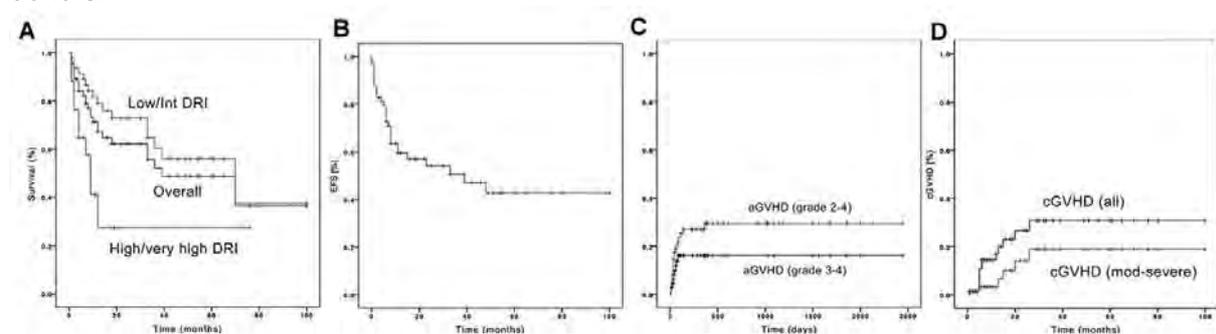
Retrospective analysis of 68 consecutive haplo HSCT performed at our centre (2008-2017). Twelve received ablative and the remainder reduced intensity conditioning (RIC). GVHD prophylaxis included post transplant cyclophosphamide, mycophenolate mofetil and tacrolimus. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined by IBMTR and NIH criteria, respectively. Event free survival (EFS) was defined as survival without relapse or disease progression. Time to event analysis was performed using the Kaplan Meier method and Cox regression used to calculate variables associated with overall survival (OS).

Results

HSCT indications included AML (n=35), lymphoma (n=13), ALL (n=9), MDS (n=6), aplastic anemia (n=3), CML (n=1), and metachromatic leucodystrophy (n=1). Thirteen received bone marrow, the remainder peripheral blood stem cells. Median follow-up was 12 months (1-100). Median OS of patients with malignant disorders was 39 months (95%CI: 8.8-69.2) (Fig 1A). 26 died, with no deaths among patients with non-malignant disorders. Among patients with malignant disorders 19 (29.7%) relapsed or progressed (median EFS 39 months, 95%CI 4.5-73.5)(Fig 1B). Frequency of aGVHD (grade 2-4) and grade 3-4 was 30% and 16%, respectively (Fig 1C). The frequency of moderate-severe cGVHD was 19% (Fig 1D). At last assessment 36 (52%) were alive, in remission and without graft rejection, and of these 24 (67%) discontinued all immune suppression. In univariate analysis factors associated with OS included: high/very high DRI (HR 3.2 96%CI 1.4-7.2, p=0.01), major ABO mismatch (HR 2.3 95% CI 1.0-5.4, p=0.05) and ablative conditioning (HR 2.9 95%CI 1.1-7.6, p=0.03). In multivariate analysis including the above three covariates only high/very high DRI remained associated with OS (HR 3.0 95%CI 1.3-7.0, p=0.01).

Conclusion

RIC Haplo HSCT offers a suitable transplantation platform for patients who lack HLA matched donors.



035. Graft versus host disease (GvHD) prophylaxis with prednisolone: a retrospective multicentre audit of outcomes.

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Background: Corticosteroids are used as first line treatment of both acute and chronic GvHD, but their role in prophylaxis is less clear.

Aim: To assess the outcome of adult patients undergoing allogeneic stem cell transplant for acute leukemia at two transplant centres in Sydney, who received prednisolone in addition to standard GvHD prophylaxis¹. Outcome measures include overall survival (OS), rates of acute and chronic GvHD, time to onset of aGvHD, relapse rates, invasive fungal infection (IFI), and cytomegalovirus (CMV) reactivation and disease.

Method: A retrospective audit of transplant databases at Royal North Shore and St Vincent's hospitals was undertaken. All transplants for AML or ALL between 2002 and 2015 who received prednisolone-based GVHD prophylaxis were included, excluding mismatched and haploidentical transplants. Analysis was done using simple descriptive statistics.

Results: Outcomes of 262 transplants, with a median follow up of 3.2 years were analysed. 260 of 262 transplants received GvHD prophylaxis with cyclosporin, methotrexate and prednisolone. The median patient age was 47 years (range 17-70). 50% had a matched related donor, 50% a matched unrelated donor.

OS was 69.4%, 64.9% and 59.9% at 1, 2 and 5 years post-transplant. 48.5% (n=127) patients developed aGvHD, with a median time to onset of 39 days. 62.6% (n=164) developed cGvHD. Relapse rates were 19% for AML, 27% for ALL. IFI were documented in 8.8% (n=23), CMV reactivation in 42.7% and CMV disease in 6.5%.

Conclusion: To the best of our knowledge, this is the largest study of outcomes following steroid-based GvHD prophylaxis to be reported. Our results suggest that the addition of prednisolone to cyclosporin/methotrexate as GvHD prophylaxis is safe and effective. OS is equivalent or superior to data for patients with AML/ALL reported to the CIBMTR during the study period. Rates of Grade II-IV aGvHD, IFI and CMV reactivation/disease appear similar to rates reported with non-steroid-containing regimens. Contemporaneous comparison with matched patients undergoing allogeneic stem cell transplant with non-steroid-based regimens is required to confirm these results.

Reference:

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036. Autologous Stem Cell Transplantation in multiple myeloma patients ≥ 65 years: single centre retrospective review

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Background: Multiple myeloma (MM) is a disease of the elderly with a median age at diagnosis of 66 years [1]. Induction chemotherapy followed by high dose melphalan ASCT is considered standard of care in patients <65 years. Historically, transplant eligibility was determined by age with limited Australian data available for ASCT in elderly MM patients. However, recent studies suggest ASCT in elderly patients is both efficacious and safe [2]. Here we report our single centre experience of ASCT in elderly MM patients.

Methods: A retrospective analysis identified 30 elderly MM patients (≥65 years) who underwent ASCT at University Hospital Geelong from 2009–2016. Data on comorbidity indices including Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) and Revised Comorbidity Myeloma Index (R-CMI), overall response rate (ORR), transplant-related mortality (TRM), progression-free (PFS) and overall (OS) survival was collected. The Kaplan-Meier method was used to estimate OS and PFS. Differences in survival between groups were assessed using the log-rank test.

Results: Median age at ASCT was 67 years (range 65-74) with 53% being males. Table 1 demonstrates baseline characteristics. 27 patients received full dose melphalan (200mg/m²). 3 received attenuated melphalan (<140mg/m²) due to renal impairment or significant previous treatment. Median follow-up was 35 months (5-82). 40% of patients (n=13) had toxicity grade III–IV (mainly gastrointestinal). TRM 3.3% (One patient died due to respiratory failure). Day 100 ORR was 83% (sCR =17%, 3/5 patients achieved MRD negativity; CR= 20%; VGPR =20%; PR=26%). Median PFS and OS were 36 and 74 months respectively (Figure 1 and 2). High-risk cytogenetics was associated with a significantly worse OS compared to standard risk (Median OS 36 months vs not reached, p<0.006)

Conclusion: Our experience demonstrates ASCT in elderly MM patients is safe and efficacious. The use of the HCT-CI and R-CMI allows identification of low risk elderly patients who are likely to benefit from ASCT, as high comorbidity scores were associated with increased toxicity, prolonged admissions [3] and inferior OS [4].

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- 3. Saad A, Mahindra A, Zhang MJ, *et al.* Hematopoietic cell transplant comorbidity index is predictive of survival after autologous hematopoietic cell transplantation in multiple myeloma. Biol Blood Marrow Transplant. 2014 Mar;20(3):402-408.
- 4. Table 1 Patient characteristics

Variable	N
ISS stage	
I	11 (37%)
II	11 (37%)
III	8 (26%)
Cytogenetics	
Standard risk	20 (67%)
High risk	8 (27%)
Insufficient cells	2 (6%)
HCT-CI score, median	0
0 (low risk)	16 (53%)
1-2 (intermediate risk)	9 (30%)
≥3 (high risk)	5 (17%)
R-CMI score, median	4
1 – 3 (fit)	13 (43%)
4 – 6 (intermediate fit)	16 (53%)
> 7 (frail)	1 (3%)
Response before transplant	
Complete remission	4 (13%)
Very good partial response	3 (10%)
Partial response	23 (77%)

037. Identification of the immune phenotypes associated with haematopoietic stem cell transplantation for Multiple Sclerosis

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Autologous haematopoietic stem cell transplant (HSCT) is an effective treatment for selected patients with severe autoimmune diseases providing a unique opportunity to study immune deregulation in humans. This study aims to elucidate the mechanisms through which HSCT achieves clinical improvement of Multiple Sclerosis (MS) in transplant recipients.

Aim and Method

We hypothesised that autologous HSCT eliminates the pro-inflammatory disease-causing T cells to halt disease progress and promotes tolerance via multiple mechanisms to sustain disease control and reconstitution of immunoregulatory suppressive cell subsets. We aim to characterise changes in these subpopulations in the peripheral blood lymphocytes (PBMC) of MS patients, being treated with HSCT at St Vincent's Hospital using four custom-designed complex flow cytometry panels with 9-14 colours including differentiation markers, chemokine receptors and trafficking markers. Analysis included *t*-test, ANOVA and tSNE.

Result

Eighteen MS patients were recruited and assessed at pre-HSCT and 3, 6, and 12 months post-HSCT time points. We found that the pro-inflammatory disease-causing T cells, CCR6+CD161hi mucosal-associated invariant T (MAIT) cells (a Th17 subset with brain-homing abilities) and CD49d+CCR6+ CNS-homing cells significantly decreased following treatment. Additionally, immunoregulatory subsets such as regulatory T cells, cytotoxic CD8+CD57+ T cells and natural killer cell subsets (CD56hi) initially increased post-HSCT and stabilised by 12 months.

Conclusion

These custom-designed flow panels successfully identified numerous characterised subpopulations in PBMC including pro-inflammatory disease-causing subsets as well as suppressive disease-stabilising subsets, combined with cell surface integrins that direct trafficking to differing tissue compartments, recently reported to be relevant to HSCT for autoimmune diseases. These findings have the potential to allow us to track disease progression and treatment outcomes by monitoring the presence of MS-causing and MS-suppressive cell types, which may help to improve the management of patients living with MS.

038 Significantly improved MUD transplant non-relapse mortality over the period of 2001-2015: an ABMTRR study

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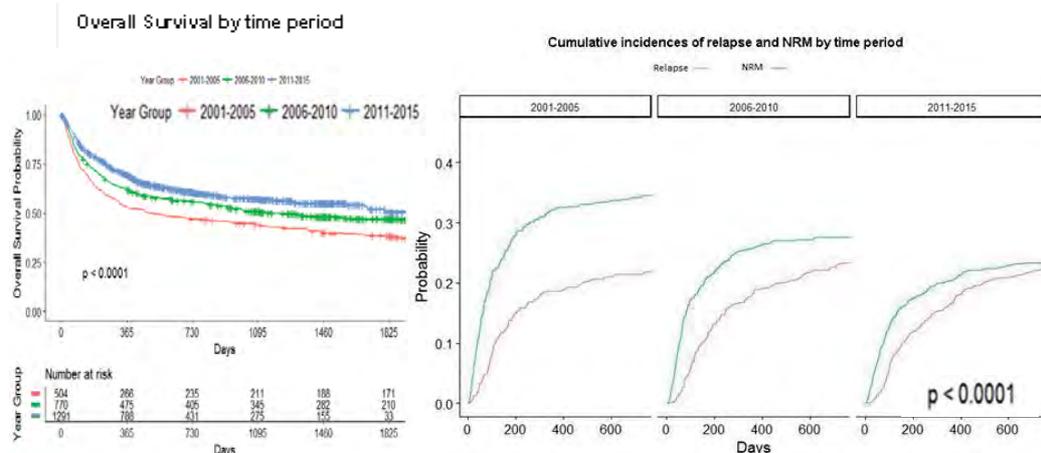
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Aim: Matched unrelated donors (MUDs) are the most common donor source for allogeneic stem cell transplantation (alloSCT) in Australia and New Zealand. Historically, non-relapse mortality (NRM) was higher compared to transplants utilising matched siblings, though recent overseas data suggest the gap is decreasing. We aimed to determine whether NRM post MUD alloSCT has reduced locally and which factors have contributed.

Method: We analysed data from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR). Adult (age >16 years) patients were included if they had received a first alloSCT utilising a MUD during 2001-2015. Data collected included patient demographics, transplant indication, transplant year, cell source, CMV serostatus, degree of HLA match, performance status, T-cell depletion, disease status and conditioning intensity. Outcome data included the incidences of disease relapse, NRM and overall survival (OS). The cohort was divided into 3 groups according to quinquennium. Differences between groups were analysed using Chi-square test, or Kruskal-Wallis for continuous variables. Probability of OS was calculated using Kaplan-Meier, while cumulative incidences of NRM and relapse were predicted using the competing risks method. Multivariate analysis using cox regression was performed.

Results: In total 2565 patients received first MUD alloSCT during the defined period. Changes over time included increasing recipient age, utilisation of PBSC, reduced intensity conditioning and T-cell depletion. Transplants occurred earlier in the disease course in later periods. Two-year OS increased from 47% in 2001-2005 to 63% in 2011-2015 ($p < 0.001$). Two-year NRM steadily decreased from 35% to 21% ($p < 0.001$) with no significant change in relapse over the same period. Factors independently associated with NRM included transplant year, recipient age, performance status, disease status at transplant, HLA match and CMV serostatus.

Conclusion: Survival following MUD alloSCT improved by almost 15% over the past decade, predominantly due to improvements in NRM.



039. Orthotopic cardiac transplant followed by autologous stem cell transplant in patients with isolated cardiac amyloidosis

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Aim

AL amyloidosis with cardiac involvement has a poor prognosis, with a median survival of less than 1 year. Autologous stem cell transplant (ASCT) is an effective therapy for AL amyloidosis, but patients with cardiac involvement are excluded from undergoing ASCT. We present a pilot study of 3 patients with isolated cardiac amyloidosis who underwent orthotopic cardiac transplant (OHT) followed by ASCT.

Methods

3 patients (2M:1F, median age 55) with AL amyloidosis with isolated cardiac involvement and Mayo stage IIIa disease were recruited. All patients received bortezomib-cyclophosphamide and dexamethasone (VCD) as initial therapy, and 1 patient had thalidomide-cyclophosphamide-dexamethasone therapy prior to VCD. OHT was performed 11, 39 and 7.2 months after diagnosis respectively. Tacrolimus and mycophenolate were used for anti-rejection prophylaxis. ASCT was performed 6.1, 6.1 and 8.3 months post OHT. Outcome measures include light chain response to combination therapy and survival.

Results

All 3 patients had elevated lambda light chains of 129, 468 and 75 mg/L prior to chemotherapy respectively. All had achieved a response to VCD chemotherapy, with 86%, 84% and 66% reduction of lambda light chains respectively. After OHT, patient 1 and 3 had grade 3A cardiac rejection, requiring increase of immunosuppression. ASCT was uneventful in all patients. One patient experienced CMV viraemia. All 3 patients have survived 11.3, 12.2 and 10.1 months post ASCT respectively. There was further light chain response in patient 1 and 3, with 97% and 87% lambda chain reduction (a complete haematological response). Patient 2 had a slight increase of lambda light chains post ASCT, but still managed an overall 68% reduction of lambda light chains, and a partial response to treatment.

Conclusion

Combined VCD, OHT and ASCT is feasible in patients who had AL amyloidosis with cardiac involvement, with clinically meaningful responses achieved in this pilot study.

040. ALCANZA: Superior brentuximab vedotin activity in CTCL across disease stages/compartments and regardless of CD30 expression

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Background: The Phase 3 ALCANZA study (*Lancet*, 2017) showed significant, durable responses with brentuximab vedotin vs physician's choice (PC: methotrexate or bexarotene) for CD30+ CTCL (mycosis fungoides [MF] or primary cutaneous anaplastic large cell lymphoma [pcALCL]). We describe ALCANZA analyses evaluating treatment response by disease stage and across disease compartments in CTCL patients, and by CD30 expression in MF patients.

Methods: Adults with previously treated CD30+ MF or pcALCL requiring systemic therapy were randomized to brentuximab vedotin 1.8 mg/kg IV, Q3W (up to 16 cycles), or PC (up to 48 weeks: methotrexate 5–50 mg PO, QW, or bexarotene 300 mg/m² PO, QD). Patients were scored CD30+ if ≥1 biopsy had ≥10% CD30+ lymphoid cells, assessed centrally. Primary endpoint was rate of objective response (ORR) lasting ≥4 months (ORR4). We compared ORR4, ORR, and complete response (CR) rate by disease stage, TNMB stage, and baseline blood disease in the CTCL population, and ORR4 and PFS in MF patients with all biopsies ≥10% CD30+ (CD30_{min} ≥10%) vs ≥1 biopsy <10% CD30+ (CD30_{min} <10%).

Results: After 22.9 months' median follow-up, ORR4 with brentuximab vedotin was greater than with PC in 97 patients with MF (50% vs 10%) and 31 with pcALCL (75% vs 20%), and consistently higher across MF disease stages. In pcALCL pts, proportions of patients with ORR4 were 89% with brentuximab vedotin vs 27% with PC in patients with skin-only disease and 57% vs 0% in patients with extracutaneous disease. In MF, ORR4 with brentuximab vedotin was greater than with PC over all CD30 expression ranges (CD30_{min} <5% [38% vs 13%], ≥5–≤20% [35% vs 10%] and >20% [76% vs 7%]).

Conclusions: Compared with PC, brentuximab vedotin produced significantly superior clinical activity across all stages and disease compartments in CTCL patients and regardless of CD30_{min} expression level in MF patients.

041. Ibrutinib versus temsirolimus (RAY study): three-year follow-up of patients with previously treated mantle cell lymphoma

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Aim

In the phase 3, randomized RAY study, ibrutinib, a first-in-class Bruton's tyrosine kinase inhibitor, demonstrated superior progression-free survival (PFS) (hazard ratio [HR]: 0.43; 95% CI: 0.32-0.58; $p < 0.0001$) (Dreyling et al. *Lancet* 2016) versus temsirolimus in patients with R/R MCL at median 20.0 month follow-up. Here we present 3-year final results.

Method

280 patients were randomized 1:1 to ibrutinib (560 mg once-daily; $n=139$) or temsirolimus (175 mg: days 1, 8, 15 of C1; 75 mg: days 1, 8, 15 of subsequent cycles; $n=141$) until disease progression/unacceptable toxicity. Long-term efficacy was investigator-assessed.

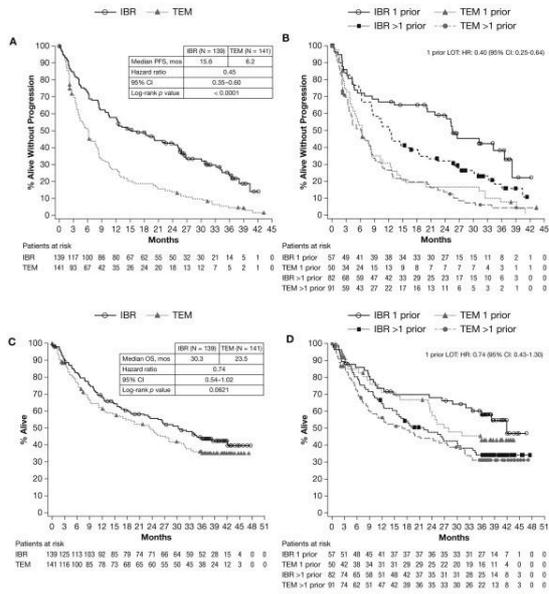
Result

At a median follow-up of 39 months, median PFS was 15.6 (ibrutinib) versus 6.2 months (temsirolimus) (HR: 0.45; 95%CI: 0.35-0.60; $p < 0.0001$) (Figure 1A); and 25.4 versus 6.2 months, respectively, for patients with one prior line of therapy (LOT) (HR: 0.40; 95%CI: 0.25-0.64) (Figure 1B). PFS2 was 26.2 versus 15.4 months (HR: 0.67; 95%CI: 0.50-0.90; $p < 0.0079$). With 39% of temsirolimus-treated patients crossing over to ibrutinib, median overall survival (OS) was 30.3 (ibrutinib) versus 23.5 months (temsirolimus) (HR: 0.74; 95%CI: 0.54-1.02; $p=0.0621$) (Figure 1C); and 42.1 versus 27.0 months for patients with one prior LOT (HR: 0.74; 95%CI: 0.43-1.30) (Figure 1D). Median treatment duration was 14.4 (ibrutinib) versus 3.0 months (temsirolimus); 24% of ibrutinib-treated and 0% temsirolimus-treated patients were on treatment at study end. Overall adverse event (AE) incidences were lower with ibrutinib versus temsirolimus. Other key efficacy and safety results are in the table.

Conclusion

These 3-year follow-up results are consistent with the primary analysis, showing clinically meaningful and significant PFS improvement for ibrutinib versus temsirolimus, with a strong trend in OS favoring ibrutinib. Patients who received ibrutinib after only one prior LOT had the most durable and best PFS and OS outcomes, supporting earlier ibrutinib use. No new safety signals were observed.

Figure 1. PFS (A) and PFS by line of therapy (B) and OS (C) and OS by line of therapy (D).



	Ibrutinib (n=139)	Temsirolimus (n=141)
Efficacy		
ORR, % ^a	77.0	46.8
CR	23.0	2.8
ORR in patients with 1 prior LOT, %	75.4	52.0
CR	33.3	4.0
Duration of response, median, months	23.1	6.3
Time to next treatment, median, months ^a	31.8	11.6
Safety		
AEs leading to treatment discontinuation, %	17.3	31.7
Grade ≥3 AEs, %	74.8	87.1
Serious AEs, %	56.8	59.7

^ap < 0.0001.

042. Frequency of perforation and impact of bowel rest among patients with aggressive non-Hodgkin's lymphoma with gastrointestinal involvement: an international, multi-centre retrospective study

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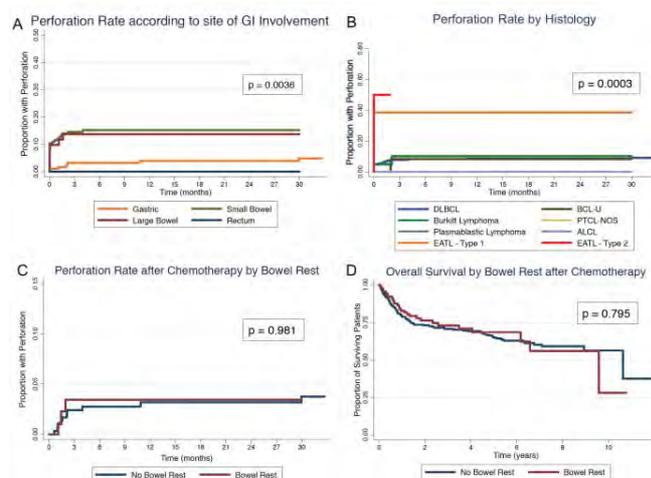
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Aim: To assess the frequency of perforation and impact of bowel rest among patients with aggressive non-Hodgkin's lymphoma (NHL) with gastrointestinal (GI) involvement.

Method: We performed a multi-centre, retrospective analysis of patients with newly diagnosed aggressive NHL with GI involvement between January 2006 and January 2016. Two out of four centers employed bowel rest as a routine measure. Survival outcomes were measured from the day of diagnosis. Uni- and multi-variate analyses of factors associated with perforation and survival were performed using Cox regression.

Results: A total of 419 patients were identified (49% treated as outpatients, 51% inpatients). Of inpatients, 106 (49%) received bowel rest. At a median follow-up of 3.6 years (range 0.1 -11.9), 41 (10%) perforated; 28 (68%) at presentation or prior to chemotherapy. Excluding these, the median time to perforation was 33 days (2 – 877). DLBCL accounted for 85% of patients (357), BCL Unclassifiable 3%, BL 5%, PTCL 2% and EATL 4%, Plasmablastic lymphoma 0.4% and ALCL 0.4%. The perforation rate varied according to site (Fig 2A) and histology (Fig 2B). By multivariate analysis, small bowel involvement (HR 3.2; 95% CI 1.4 – 7.3, P=0.005), large bowel involvement (HR 3.2; 95% CI 1.2 – 8.9, P=0.026) and EATL (HR 3.3; 95% CI 1.4 – 7.7, P=0.007) were associated with increased perforation risk. In the patients who did not perforate prior to commencing chemotherapy, bowel rest was not associated with differences in rates of perforation (3.3% v 3.4%, P=0.981; Fig 2C), peritonitis (100% v 50%, p=0.231), surgery (100% v 90%, P=0.990) or overall survival (HR = 0.95, 95% CI 0.6-1.5, P=0.795; Fig 2D)

Conclusion: Perforation occurs in approximately 10% of patients with aggressive NHL and GI involvement. These data do not support a benefit for bowel rest and TPN in the management of unselected patients with aggressive NHL and GI involvement.



043. Excellent Outcomes of Older patients with PCNSL using R-MPV/Ara-C immunochemotherapy without Whole Brain Radiotherapy (WBRT)

Tatarczuch M¹, Gilbertson M¹, Gregory G¹, Opat S¹

¹Monash Health, Melbourne, Australia **BACKGROUND**

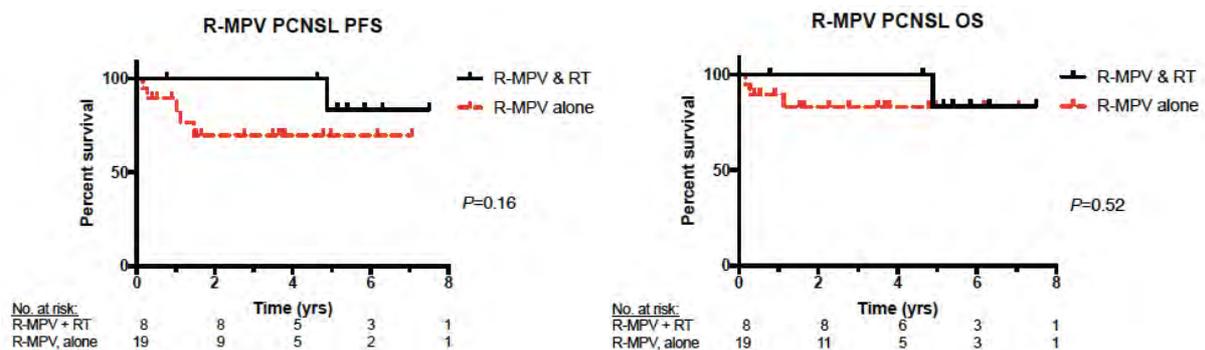
The optimal strategy for Primary CNS Lymphoma (PCNSL) is undefined due to its rarity and difficulty enrolling patients in clinical trials. WBRT is associated with unacceptable neurocognitive toxicity in older patients. Furthermore, recent studies of intense regimens and Autologous Stem Cell Transplantation exclude older patients with comorbidities. IELSG32 confirmed the benefit of combining rituximab to intense regimens, albeit in a younger cohort. This study seeks to examine the role of rituximab and WBRT in older PCNSL patients.

Method

Patients presenting to Monash Health, October 1997-December 2016 with PCNSL (WHO Criteria: 2008) and treated with curative intent using (R)-MPV/Ara-C (Morris PG et al. J Clin Oncol. 2013;31(31):3971-9.) were identified. Only cases with adequate information i.e. baseline characteristics, treatment and outcome were included. Outcome analysis was restricted to the rituximab cohort. Overall survival (OS) and progression free survival (PFS) were modelled by Cox regression.

Result

43 patients were identified, with 27 receiving rituximab. In the rituximab cohort, 20 were >60yo and 8 received consolidative WBRT. Patients who did not receive WBRT were older (median 72y v 51y p<0.001), however had similar IELSG risk. Three patients died prior to completion of induction: progressive disease (1), sepsis (1), neurological complications (1). 24/27 patients were assessable for response and all achieved CR. Use of rituximab was associated with superior PFS and OS in older patients: 5yr PFS 86% v. 17%, HR 0.32, P=0.099; OS (88% v. 17% HR 0.22, P=0.019; Despite being older, patients who did not receive WBRT had similar outcomes to those that did: 5yr PFS 70% v. 83%, HR 0.36, P=0.16; OS (83% v. 83%, HR 0.49, P=0.52; (Figure 1 A & B). Neurotoxicity was reported in 3/8 patients receiving WBRT and 2/16 who didn't (p=0.32).



Conclusion

Older patients treated with R-MPV/Ara-C without WBRT have excellent outcomes, comparable to younger patients. Age and comorbidity should not exclude patients from receiving potentially curative immunochemotherapy.

044. Transformed Lymphomas: an Australian Tertiary Centre Experience

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Aim

Transformation of indolent lymphomas into aggressive histology has historically been associated with a poor prognosis. With the development of new treatments in the last decade, our present study examined the natural history of transformed lymphomas in the modern Australian setting. Transformation of FL, as well as less-described indolent lymphomas (eg. MZL, LPL) were included. Richter's transformation, a distinct entity with known poor outcome, was excluded.

Methods

We performed a 20-year retrospective study (1 July 1998 – 31 June 2017) of patients with biopsy-proven transformed lymphoma identified from hospital databases. Cases with adequate information on baseline characteristics, treatments received and clinical outcomes were included. OS/PFS were constructed using the Kaplan-Meier method. Associations between clinical factors and OS/PFS were evaluated using Cox proportional hazards model.

Results

A total of 72 patients were identified, with a median age at diagnosis of 68.1 (range: 18.5, 86.8) and of which 34 (47%) were male.

Histology prior to transformation were 50 FL (70%) and 22 others i.e. non-FL (30%; including 16 MZL, 2 LPL and 4 other indolent lymphomas).

69/72 (96%) patients were assigned to the 'poor' (n=41, 57%) or 'good' (n=28, 39%) R-IPi group.

The majority of patients (n=50, 69%) received R-CHOP or R-CHOP-like regimen. Induction responses were comparable to primary nodal DLBCL with a CR of 72%.

At analysis, there were 16 relapses (22%) and 24 deaths (33%, 8 from relapsed disease) in total.

With a median follow-up of 2 years of this cohort, OS and PFS were 67% and 56%, respectively.

There is significantly less relapse (n=1, 5%) in transformed non-FL subgroup vs. transformed FL (n=15, 30%). However, only 7 (46%) patients with relapsed transformed FL subsequently died.

Conclusion

In contrast to previously published studies, the clinical outcomes of biopsy-proven transformed lymphomas (OS/PFS) were more favourable in our case series. Additionally, a superior PFS of transformed non-FL vs. transformed FL was noted, although their OS were comparable

045. Outpatient-based immunochemotherapy is associated with favourable survival for patients with primary mediastinal large B-cell lymphoma

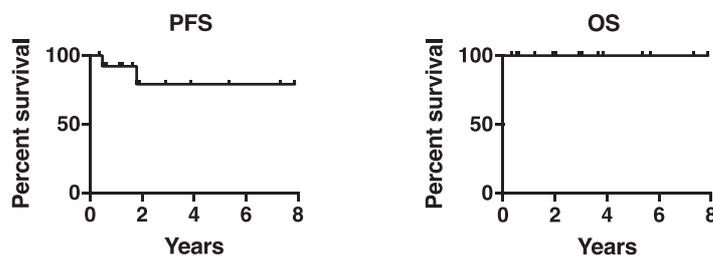
Liu F¹, Shortt J^{2,3}, Gilbertson M², Fedele P², Grigoriadis G^{2,3}, Low M², Ratnasingam S², Vilcassim S^{2,3}, Patil S^{2,4}, Opat S^{2,3}, Gregory G^{2,3}

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Aim: Primary mediastinal large B-cell lymphoma (PMBCL) is a rare and distinct clinicopathologic and molecular entity of non-Hodgkin lymphoma arising from thymic B-cells. Historical retrospective series have demonstrated superior OS with 3rd generation protocols such as doxorubicin, cyclophosphamide, vincristine, methotrexate, bleomycin and prednisolone (MACOP-B) over R-CHOP-based therapy¹. We retrospectively assessed outcomes of patients uniformly treated at our institution with rituximab in combination with MACOP-B (R-MACOP-B).

Method: Retrospective study of adult PMBCL cases treated at our institution from 2006-2017 was performed from using an institutional database and electronic medical records in accordance with institutional ethics approval. GraphPad Prism software was used for statistical analysis.

Results: Seventeen adult patients with PMBCL were identified. Due to initial diagnostic uncertainty or presentation *in extremis* three patients were initially managed with R-CHOP and were excluded from the analysis. Of the remaining 14 patients median age at diagnosis was 36 years (19-63), and majority were male (10). All patients had bulky mediastinal disease with median long-axis diameter 12cm (range 10-20.3) and were Ann-Arbor stage I-II consistent with PMBCL classification criteria. All patients completed R-MACOP-B with dose-limiting toxicity prior to completion in 2 patients [omission of vincristine (n=2) and dose-reduction of methotrexate (n=1)]. Consolidative radiotherapy was administered to 11 patients. At median follow-up of 3 years (range 0.4-7.8), PFS was 86% (n=12) and OS was 100%. One patient achieved PR as best response to R-MACOP-B and was successfully salvaged (schedule unknown) after relocating internationally and one patient relapsed at 1.8 years and was successfully salvaged with IVAC x 2 and autologous stem cell transplantation. No deaths related to disease or treatment were observed.



Conclusion: R-MACOP-B remains an acceptable outpatient-based front-line therapy for patients with PMBCL as our experience demonstrates excellent survival outcomes comparable to published historical data.

Reference: Savage KJ et al. Ann Oncol. 2006; 17:123-30.

046. Novel combination therapies with the Pol I inhibitor CX-5461 significantly improve efficacy in multiple myeloma

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Background: patients with multiple myeloma (MM) who relapse after initial treatment continue to have a dismal prognosis. MM is a highly heterogeneous disease, confounding targeted therapy design. Further, single agent therapy rapidly leads to acquired resistance, indicating combination therapy is essential.

Our laboratory developed CX-5461, a highly selective small molecule inhibitor of ribosomal gene transcription, currently in a phase 1 trial in relapsed/refractory haematological malignancies (Peter Mac). CX-5461 inhibits RNA polymerase I (Pol I) transcription, eliciting a highly specific DNA damage response (DDR), killing malignant cells while sparing normal cells^{2,3}. We demonstrated that single-agent treatment with CX-5461 provides a significant survival benefit in murine models of B-cell lymphoma, acute myeloid leukaemia and MM³⁻⁵. However, drug resistance eventually occurs.

Aim: to examine the efficacy of CX-5461 in combination with other agents having proven clinical or promising preclinical efficacy in MM.

Methods: we conducted a boutique, high-throughput screen in myeloma cell lines of CX-5461 combined with a targeted range of agents. We measured the effect of CX-5461 on proliferation, cell death, cell cycle distribution, DDR biomarkers and on-target effects. The effect of combining CX-5461 with one candidate, the histone deacetylase inhibitor (HDACi) panobinostat, was tested in vivo using the tVκ*MYC murine model of MM⁶.

Results: CX-5461 showed increased anti-proliferative effect and cell death in combination with multiple drug classes. The proteasome inhibitor carfilzomib and the HDACi panobinostat demonstrated the most impressive synergy with CX-5461. Using the tVκ*MYC model, we showed that CX-5461 with panobinostat provides a significant survival advantage. We are currently interrogating the molecular synergistic response of this combination, including p53-dependent and -independent responses, and the ATM/ATR signalling pathway.

Conclusion: CX-5461 shows increased efficacy when combined with multiple drug classes, with panobinostat and carfilzomib showing the most promise. These results will direct the subsequent clinical trial using combination drug therapy.

Drygin et al., Cancer Research 2011

1. Quin et al, Oncotarget, 2016
2. Bywater et al., Cancer Cell 2012
3. Devlin et al., Cancer Discovery 2016
4. Hein et al., Blood 2017
5. Chesi et al., Blood 2012

No conflict of interest to disclose

047. Circulating tumour DNA analysis in myeloma reveals a dominance of mutations in DNA-repair genes

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Aim

Multiple Myeloma (MM) is a multi-focal malignancy currently relying on single-site bone marrow (BM) biopsies for mutational characterisation, which fails to capture the spatial and temporal genetic complexity. We have recently established that cell-free tumour DNA (ctDNA) can be utilised to address the shortcomings of BM biopsy for mutational characterisation.

Method

Paired BM MM cell DNA and plasma (PL) ctDNA from 82 patients were analysed for 4 genes (KRAS, NRAS, BRAF and TP53 covering 65% of mutations in MM) utilising the highly-sensitive (0.001%) OnTarget™ Mutation Detection (OMD) platform. Targeted deep sequencing (TS) of a 23-gene custom panel with lesser sensitivity than OMD (0.01%) but covering 95% mutations was performed for ctDNA from 29 patients of which 15 had matched BM sample.

Results

OMD revealed that 28% of patients harboured PL-specific mutations in addition to mutations also detected in the BM and that in 14% of patients, mutations were present only in the PL with none detected in the BM. Activating RAS mutations were highly prevalent with 65% harboring at least one RAS mutation, with ≥ 3 RAS mutations in 14 patients, indicating a striking sub-clonal convergence on this pathway. Notably, no mutations were detected in either BM or PL in 24% of the patients. Subsequently, TS of 23 MM-specific genes revealed the presence of mutations in 93% of patients with 60% harbouring more cancer-associated genes in the PL than BM. Additionally, while RAS/RAF mutations were predominant in the BM (60%), ctDNA had a significantly higher proportion of mutations in DNA-repair genes ATM, ATR and TP53 (31% in RAS/RAF vs 58% in DNA-Repair, $p < 0.008$).

Conclusion

Our ctDNA analyses indicates the existence of mutant clones present predominantly or exclusively distant to BM biopsy sites in a significant number of patients. Furthermore, ctDNA analysis has revealed a mutational spectrum dominated by DNA-repair genes.

048. Cell-free DNA: a promising biomarker for monitoring tumour burden in multiple myeloma

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Aim

ROAR is a phase 1b trial of oral azacytidine with lenalidomide and dexamethasone for relapsed and/or refractory multiple myeloma (RRMM). We have previously demonstrated that interrogation of plasma (PL) cell-free DNA (cfDNA) enables more comprehensive mutational characterisation of MM than bone marrow (BM), while allowing repeated sampling to define changes in tumour burden. In this study, we evaluated the utility of cfDNA for the characterisation and monitoring of tumour burden in a homogeneously treated MM cohort.

Method

Sequential PL cfDNA (baseline, day 5, end of cycles 3 and 6) were extracted from 26 patients on study. 21 were subsequently screened for KRAS, NRAS, BRAF and TP53 mutations using the OnTarget™ Mutation Detection (OMD) platform. cfDNA levels were compared to conventional serum biomarkers (paraprotein and serum-free light chains [SFLC]). The fractional abundance (FA) of mutations in sequential PL samples were correlated to clinical outcomes.

Results

- Median concentration of cfDNA was 26.2ng/ml of plasma correlating with copies/ml of the RPP30 gene as determined by ddPCR (R square 0.88, p<0.01).
- cfDNA concentration and/or RPP30 correlated with SFLC and/or paraprotein in 70% of patients.
- OMD detected 53 mutations (PL= 19, BM= 14, both=20) in 17/21 (81%) patients.
- TP53 mutations were predominantly PL-only (56%), consistent with our previous description of genetic spatial heterogeneity in RRMM.
- Tracking of sequential PL for mutant clones revealed increasing FA coincident with relapse in three patients; one patient had FA increase prior to serological relapse and the fifth patient showed no correlation.
- Differential clonal responsiveness to therapy was observed in the two patients with multiple mutations.

Conclusion

These data confirm that cfDNA can provide more comprehensive and quantifiable mutational information in MM than BM evaluation. Sequential tracking of cfDNA may be useful to predict disease relapse and this will be further analysed in this annotated sample set.

049. The IMiDs, through loss of Ikaros and Aiolos, derepress CD38 and functionally synergise with Daratumumab

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Background

The transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) have recently been found to be targeted for destruction by the immunomodulatory drugs (IMiDs). However, the role of Ikaros and Aiolos and why their loss leads to MM cell death remains unclear.

Methods/Aim

We have used CRISPR-Cas9 genome editing to delete IKZF1 and IKZF3 in human MM cell lines to gain further insight into their downstream gene regulatory networks.

Results

Consistent with the action of the IMiDs, loss of either of these genes resulted in both a G1/S cell cycle arrest and induction of apoptosis. This was not dependent on the subsequent reduction of the IRF4-MYC “axis”, as neither were consistently downregulated and retroviral expression failed to rescue Ikaros loss. This led us to further investigate the transcriptional changes resulting from loss of Ikaros, Aiolos or treatment with lenalidomide using RNA-sequencing. This data will be discussed in the presentation.

Importantly both Ikaros and Aiolos were found to repress the expression of interferon stimulated genes (ISGs) and their loss lead to activation of an interferon-like response, possibly contributing to cell death. In keeping with this, treatment with lenalidomide and low dose IFN β resulted in synergistic cell death. Furthermore, CD38 appears to be both an ISG and repressed target of Ikaros/Aiolos, and its expression is increased at both mRNA and surface expression on their loss. Given recent clinical studies have shown improved outcomes with combination treatment with lenalidomide and the anti-CD38 monoclonal antibody Daratumumab, we wondered whether this represented a MM cell intrinsic mechanism explaining this synergy. Consistent with this hypothesis, loss of Ikaros, or treatment with lenalidomide or IFN β lead to increased daratumumab induced NK cell mediated antibody-dependent cellular cytotoxicity.

Conclusion

These results give further insight into the mechanism of action of the IMiDs, and provide mechanistic rationale for combination with anti-CD38 monoclonal antibodies.

No conflict of interest to disclose.

050. Demethylation sensitises t(4;14) multiple myeloma to Ras-MAPK pathway inhibition

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Aim

The t(4;14) translocation in 15% of multiple myelomas (MM) juxtaposes the MMSET/FGFR3 locus on chromosome 4 and the IgH gene on chromosome 14 leading to dysregulation of both MMSET and FGFR3 and defines a subset of patients with shortened survival. FGFR3 is a transmembrane growth factor receptor that inputs into the Ras-MAPK pathway. FGFR3 overexpression via t(4;14) is considered mutually exclusive with activating Ras mutations (Ras^M). We investigated the impact of trametinib (MEK inhibitor) ± azacitidine (AZA) pre-treatment (hypomethylating agent) on human myeloma cell lines (HMCLs) harbouring t(4;14) or neither t(4;14) nor Ras^M (WT).

Methods

Three t(4;14) HMCLs with FGFR3 overexpression (19-500 fold overexpression c/w WT HMCLs) and 2 WT HMCLs were pre-treated with AZA (200nM) daily for 7 days followed by trametinib (10nM, 100nM and 1µM) for 72 hours. Proliferation, cell viability and cell cycle were evaluated by cell count and propidium iodide based flow cytometry. RNA was isolated from t(4;14) cells post AZA treatment for RNA sequencing using the HiSeq2500 platform to define gene expression changes that impact on sensitivity to MEK inhibition.

Results

Neither the AZA (200nM) pre-treatment nor single agent trametinib had any effect on either group of HMCLs in all outcomes measured. Conversely trametinib exposure following AZA pre-treatment resulted in a significant reduction in proliferation ($p < 0.03$) in all t(4;14) HMCLs at all doses. The most profound reduction in proliferation of 55-92% ($p < 0.03$) was seen at 1µM. Similarly following trametinib (1µM) a relative increase of 25-55% of cells in the pre-apoptotic phase of the cell cycle was observed in 2 of 3 t(4;14) HMCLs. Interestingly, no significant changes in all outcomes measured were observed in WT HMCLs despite AZA pre-treatment.

Conclusions

The combination of azacitidine and trametinib is effective against t(4;14) MM representing a potential targeted novel therapeutic approach that warrants further evaluation.

051. Macropinocytosis as an important route of tumor nutrient uptake in KRAS-mutated myeloma cells

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Aim

Sustained cancer cell growth requires up-regulation of nutrient acquisition mechanisms. Novel approaches can identify “tumor fuel”, the way it is obtained and then altered for cellular usage ^(1, 2). This study investigated the role of macropinocytosis as a protein uptake mechanism in myeloma cells.

Method

Macropinosomes were visualized using confocal microscopy in KRAS-mutated and WT human myeloma cells utilising TMR-dextran and 5-[N-ethyl-N-isopropyl] amiloride (EIPA) as a marker and specific inhibitor of macropinocytosis, respectively. Internalization of labelled albumin and its co-localization with TMR-dextran were evaluated in parallel. Degradation of macropinocytic albumin was evaluated by dual labelling of cells with TMR-dextran and DQ-BSA. Cell sensitivity to glutamine deprivation was examined with an ATP-based viability test. To evaluate the possible correlation of macropinocytosis to cell phenotype, macropinocytosis in WT-KRAS TK1 (intra-medullary) and WT-KRAS TK2 (leukaemic) cells contemporaneously derived from the same patient was studied.

Result

KRAS-mutated KMS28-PE, KMS18 and MM1S cells displayed higher levels of macropinocytosis compared to WT-KRAS KMS34, KMS12-BM and TK1 cells. This was inhibited by EIPA. Macropinocytosis led to the internalization of albumin which subsequently underwent proteolytic degradation. In sub-physiological concentrations of glutamine the viability of WT-KRAS cells was reduced when compared with KRAS-mutated cells that not only remained viable but could continue to proliferate over a period of 7 days ($p < 0.05$). Albumin rescued the partially compromised growth of KRAS-mutated cells at sub-physiological glutamine concentrations and this effect was abrogated by EIPA. Interestingly, the leukaemic TK2 cells but not the marrow-derived TK1 cells demonstrated high levels of macropinocytosis comparable to mutated-KRAS cells.

Conclusion

Macropinocytosis is a mode of protein uptake in KRAS-mutated and leukaemic-phase myeloma cells that maintains cellular viability in the context of nutrient deprivation. We hypothesise that enhanced macropinocytosis may promote a more ‘metastatic’ phenotype and that pharmacological inhibition of macropinocytosis may represent a novel therapeutic approach for myeloma.

052. PET- directed therapy in Follicular Lymphoma

Trotman J

Aim and Method

Standardised lymphoma staging and response assessment facilitates exchange of information, guiding prognosis and therapeutic decision making. The 1971 Ann Arbor system provided such guidance until a pivotal shift came in 2007 with the recognition that PET-CT is central to staging of most FDG-avid lymphomas. In the Lugano 2014 criteria the central role of PET in staging and response assessment for follicular lymphoma (FL) was formally acknowledged.

More recently, studies suggest that baseline Total Metabolic Tumour Volume (TMTV) on PET may more accurately quantify tumor burden for determining prognosis. With standardisation we may be getting closer to a single staging parameter, 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.

Results

Independent postinduction PET assessment in four studies (PRIMA, FOLL05, PET FOLLICULAIRE and GALLIUM) have confirmed the impact of achieving complete metabolic remission (CMR, Lugano 2014) on both PFS and OS after frontline therapy of FL. Outcomes for the majority ~80% of patients who achieve CMR after induction therapy prompt consideration to PET-directed de-escalation of therapy in this population. Conversely the poor prognosis of those who remain PET-positive provides the impetus for study of escalation or change in therapy.

Conclusion

TMTV has the potential to provide the single most efficient and relevant means of informing clinicians and patients of their disease burden at diagnosis, and combined with postinduction PET staging and response assessment, may become a new standard to convey prognosis and rationale for study of tailored therapy in FL. The current RePLY and upcoming PETReA studies offer the potential to improve the poor prognosis of patients who remain PET-positive.

053. Aggressive Lymphoma

Sehn L
University of British Columbia

Abstract not provided

054. Hodgkins Lymphoma

Johnson P

Aim and Method

Hodgkin's lymphoma can be very effectively treated using chemotherapy, sometimes with radiotherapy, and recent studies of both early and advanced lymphoma have recorded fewer deaths from the disease than from other causes. For young patients with a high chance of disease-free survival, the long-term side effects are an important consideration, and there is a need to balance the early benefits of increased treatment intensity against harder-to-record delayed harms such as infertility, second malignancies, heart disease and other damage. Baseline clinical features are of limited predictive utility in this respect, so a number of trials have tested functional imaging with ¹⁸FDG-Positron Emission Tomography during the course of treatment to assess the response and modulate subsequent therapy accordingly. An important development facilitating this has been the reproducible definition of metabolic response according to a standardised 5-point scale, the Deauville score.

Results

In early stage disease, the results show that omission of consolidation radiotherapy following a good response to ABVD chemotherapy can be done without detriment to overall survival, despite a small increase in rates of recurrence, of the order of 5%. For those with poor responses, escalation to more intensive BEACOPP chemotherapy appears an effective strategy, with improved disease control even when consolidation radiotherapy is also given.

In advanced disease, a number of trials have started treatment with ABVD, with escalation to BEACOPP or even myeloablative therapy for patients remaining PET-positive after 2 cycles, yielding durable remissions in around 65%, compared to less than 30% in historic series. De-escalation by omission of bleomycin and consolidation radiotherapy after a negative interim PET scan appears safe, with no increase in the recurrence rate, although the negative predictive value of interim PET after ABVD is sub-optimal, especially for those with very advanced disease at presentation, with recurrence rates in stage IV disease after a negative scan around 20%. The emergent results show that the negative predictive value of PET is higher after escalated BEACOPP chemotherapy, although the positive predictive value appears poor, and addition of rituximab for patients interim PET positive after BEACOPP does not improve the outcomes. The approach of initial BEACOPP, de-escalating to ABVD for those with negative interim PET shows promising early results and may be a preferable strategy for those presenting with the highest risk disease.

Conclusion

Response-adapted therapy has yielded important results for patients with Hodgkin lymphoma and is becoming established as a standard approach, which will support the introduction and testing of new agents such as brentuximab vedotin and anti-PD1 antibodies.

055. CAR T-cells in ALL

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The recent approval of CAR T-cells for treatment of paediatric B-cell acute lymphoblastic leukaemia (B-ALL) by the US Food and Drug Administration (FDA) is an inflection point in the treatment of haematological malignancy. This approval by the FDA is in the wake of outstanding results seen in multiple clinical trials. However significant challenges to widespread incorporation of CAR T-cells into the management of B-ALL remain. These include cost, logistics of production and distribution, and immediate and long term safety concerns. The use of novel gene modification systems in simplified production protocols, combined with clinical trial design taking into account the interaction between cell dose and tumour bulk will enable the broader use of CAR T-cells for B-ALL. This in turn will provide a platform for further CAR T-cell refinement, enhancing their efficacy and safety, hopefully leading to replacement of more toxic therapies such as intensive chemotherapy and allogeneic stem cell transplantation.

056. Management of ALL - prognostication, MRD, and indications for SCT

Gökbuget N
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Abstract not supplied

057. New therapies in ALL

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Abstract not supplied

058. AML induction outcomes in two different eras: analysis of ALLG AMLM7 and AMLM12

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Aim

To explore the outcome differences after AML induction therapy using ICE (idarubicin 9 mg/m² d1-3, cytarabine 3 g/m² d1,3,5,7, and etoposide 75 mg/m² d1-7) from ALLG AMLM7 and AMLM12 studies.

Methods

AML M7 and AML M12 were conducted between 1995-2000 and 2003-2010 using the same induction chemotherapy (ICE) backbone. For this analysis, AML with favourable karyotype was excluded. 256 patients from AML M7 were matched 1:1 to AML M12 using propensity score matching. Time-to-event analyses were censored for allogeneic transplant or lost to follow-up. Logistic regression was used to identify predictors of 30-day mortality. Analyses were performed using R version 3.3.2.

Results

Baseline characteristics are shown in [Table](#). Survivors from AML M7 and AML M12 were followed for a median of 42.8 and 49.3 months, respectively, with similar leukaemia-free survival (median 12.8 vs 12.6 months). Overall survival (OS) for patients in AML M12 was better than those in AML M7 (median not reached vs 53.6 months, p=0.006) ([Figure](#)). However, the OS was similar after limiting the analyses to patients who underwent randomisation to consolidation chemotherapy. To explain the discrepancy, 30-day mortality was examined, revealing significantly higher early deaths in AML M7 than AML M12 (11.3% vs 3.5%, p=0.001). After multivariate analysis, participation in the AML M7 cohort remained the most significant risk factor for 30-day mortality (OR 3.14), whereas other significant risk factors were balanced between the cohorts: age (OR 1.08 for each additional year), ECOG >0 (OR 1.77) and WCC (OR 1.06 for each additional 10 x 10⁹/L). Aspects of supportive care were examined: mould-active antifungal prophylaxis became available in AML M12, whereas transfusion support (both red blood cells and platelets) and infection rates (both clinical and microbiological) were similar.

Conclusion

Better supportive care with a reduction in early mortality has allowed the ICE induction regimen to be safely administered to AML patients in the modern era.

059. Analysis of publicly available datasets reveals the intersect of age and molecular subtypes in acute myeloid leukaemia

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Genetic profiles predict clinical outcomes in chemotherapy-treated AML. The 2017 update of the European LeukemiaNet recommendations on genetic risk stratification (ELN2017)¹ provides the most comprehensive genomic classification of any tumour subtype. We sought to evaluate ELN2017 compared to ELN2010 and understand the intersect between genomics and clinical variables.

Methods We systematically reviewed published AML datasets and identified three large cohorts with sufficient genomic and clinical data to calculate ELN2017. These comprised 2409 AML patients (AMLSG², n=1,316; TCGA³, n=150 and TARGET⁴ paediatric AML n=943). Comparisons between groups were performed using Mann-Whitney-U-test for continuous or X²-test for discrete variables. Kaplan-Meier survival curves were compared using a log-rank test.

Results ELN2017 predicted survival in all datasets, however did not define a favourable risk subgroup in TCGA. A key difference between ELN2017 and ELN2010 favourable risk is the inclusion of NPM1+ with low allelic ratio FLT3-ITD (NPM1+ITD-L), compared to intermediate-risk in ELN2010. In AMLSG, NPM1+ITD-L patients had inferior survival compared with NPM1+ITD- or other favourable risk patients (1-year overall survival (OS) 61% vs. 84% vs. 91%, p<0.001). OS in NPM1+ITD-L overlapped ELN2017 intermediate risk patients (1-year OS 70%) (Fig.1). The TCGA cohort showed similar OS for NPM1+ITD- and NPM1+ITD-L, both having poor survival (1-year OS 50% vs 58%, p=0.8). In TARGET, NPM1+ITD- and NPM1+ITD-L patients had similar, favourable survival profiles. Given the prognostic impact of age, we examined survival in age <60 vs ≥60 for each adult cohort. In age <60, NPM1+ITD-L had inferior survival compared to NPM1+ITD- patients. In age ≥60 yrs, both subgroups had dismal outcomes (Fig.2).

Conclusion These data demonstrate the prognostic capability of ELN2017, however raise important caveats. NPM1+ITD-L patients have survival inferior to other favourable risk patients and more similar to intermediate risk cohorts. Furthermore, patients ≥60 years with NPM1 mutations, with or without FLT3-ITD, have adverse outcomes and should not be considered favourable risk.

References 1. Döhner, et al. 2017, *Blood*. 2. Papaemmanuil, et al. 2016, *NEJM*. 3. TCGA, 2013, *NEJM*. 4. Farrar, et al. 2016, *Cancer Research*.

*VL and JS contributed equally to the study. VL is supported by an NHMRC postgraduate scholarship. SWL is supported by a CSL Centenary Fellowship, NHMRC, Cancer Australia/ Cure Cancer Australia and gratefully acknowledges the support of the Gordon and Jessie Gilmour Leukaemia Research Trust.

060. Outcome after high vs intermediate-dose cytarabine in combination with idarubicin as induction therapy in AML

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Aim

The optimal cytarabine dose in induction chemotherapy regimen for adult AML remains contentious. We examined the outcomes of younger AML patients treated with idarubicin (12mg/m² d1-3) in combination with high-dose (3g/m² BD d1,3,5,7; HDAC3) or intermediate-dose (1.5g/m²; IDAC3) cytarabine.

Methods

Consecutive AML patients age ≤60 years at the Alfred and Austin Hospitals were treated with HDAC3 (n=38; 2010-2014) or IDAC3 (n=35; 2014-2017). Consolidation chemotherapy is IcE (idarubicin 9mg/m² d1-2, cytarabine 100mg/m² d1-5, and etoposide 75mg/m² d1-5) for up to 2 cycles. Favourable-risk karyotypes were excluded. We assessed treatment responses (2017 ELN Recommendations), early mortality (30 and 60 days), delivery of consolidation therapy (days from start of induction therapy and number of cycles), rates of allogeneic transplant in first remission, and overall (OS) and leukaemia-free survival (LFS) censored for allogeneic transplant or lost to follow-up. R version 3.3.2 was used.

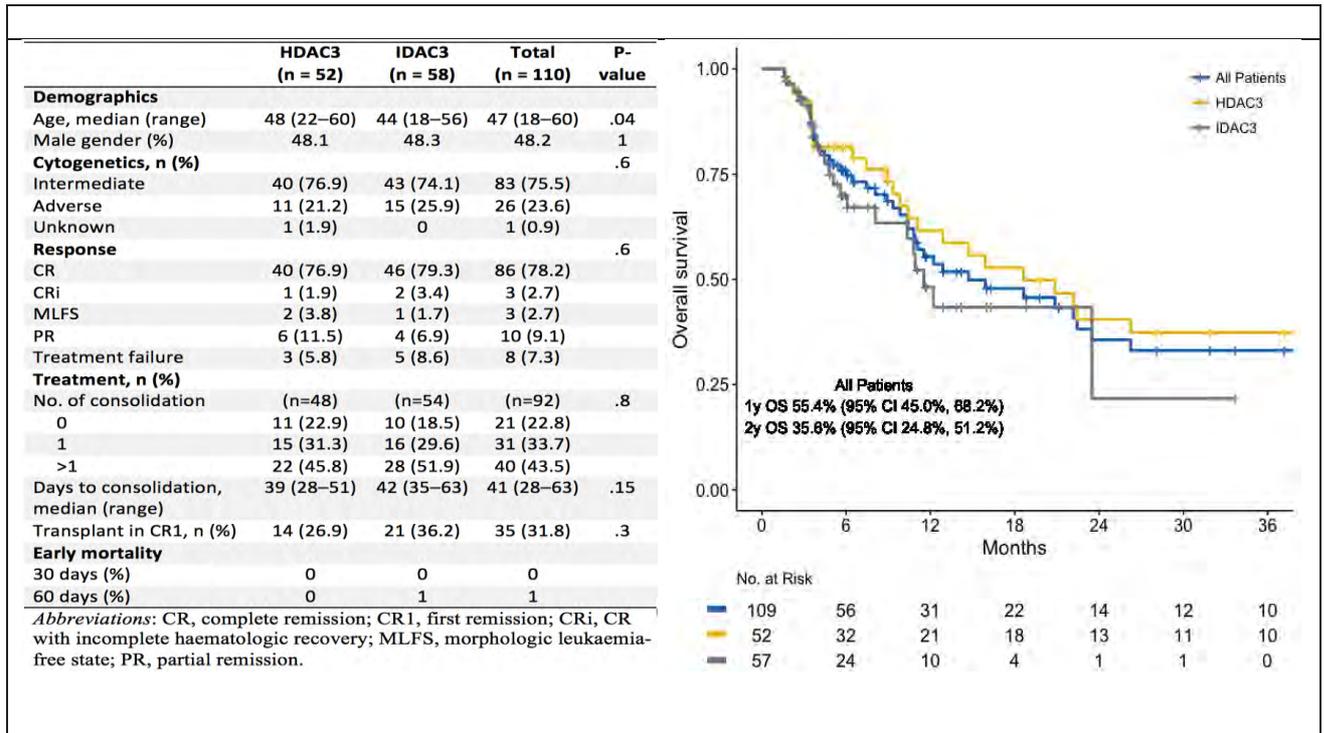
Results

IDAC3-treated patients were younger than HDAC3 (median 44 vs 48 years). Overall, 76% were intermediate-risk karyotype. Survivors were followed up for a median of 8 (IDAC3) and 37 months (HDAC3), respectively. No significant differences were observed for any outcome ([Table](#)). Overall CR/CRi rates were 81% after one induction, with only one death at 60 days. Delivery of consolidation therapy was similar for both groups, including allogeneic transplant in first remission; 9/15 (60%) in IDAC3 and 7/11 (64%) in HDAC3 among adverse-risk cytogenetics. Median OS were 11.5 and 18.6 months for IDAC3 and HDAC3 (p=0.3), respectively ([Figure](#)), with the corresponding median LFS of 10.9 and 9.4 months. Data on *NPM1*-mutant minimal residual disease and mucositis will be presented at the HAA meeting.

Conclusion

IDAC3 and HDAC3 are both well tolerated in younger AML patients with high first-cycle CR/CRi rates and low early mortality. With the caveats of limited sample size and follow-up duration, survival outcomes appeared equivalent to each other.

Table. Summary patient and disease characteristics. **Figure.** Overall survival of AML patients treated with IDAC3 and HDAC3 induction chemotherapy.



061. Long-Term Outcomes of Low-Dose Cytarabine/Thioguanine Based Metronomic Chemotherapy (STIMULus) in Elderly Patients with AML

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Aim

Elderly patients with AML experience increased complications of therapy, lower complete response rates, shorter remissions and shorter median survival relative to younger cohorts. This prognostic disparity necessitates the investigation of novel therapeutic approaches relevant to elderly patient populations. 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 55 patients with AML (37 newly diagnosed, 18 relapsed or refractory) treated with the outpatient-based metronomic protocol of subcutaneous cytarabine 20mg/m² and oral thioguanine 40mg/m² given for 14 – 21 days in monthly cycles between 2008-2016 (STIMULus). The primary outcome measure was complete response rate with secondary outcome measures of overall survival and leukaemia free survival.

Results

The study cohort was a high risk group of median age 75 years (52.8 – 94.1) consisting of 32 patients (58.2%) with secondary AML, and 23 patients (42%) with adverse or intermediate cytogenetic risk. The median bone marrow blast count was 51% (8 – 98%). Overall, a CR/CRi was achieved in 33/55 patients (60%). In the subset of patients with newly diagnosed AML 28/37 patients (75.7%) achieved CR/CRi with 5/18 patients (27.8%) of patients with relapsed or refractory disease achieving CR/CRi. Median overall survival across all patients was 302 days (95% CI; 96 – 508 days). The median overall survival in newly diagnosed patients was 456 days (95% CI; 188 – 724 days) vs. 166 days (95% CI; 72 – 260 days) in patients with relapsed or refractory disease (P = 0.150). Leukaemia free survival was 485 days (95% CI; 36 – 934) across the entire patient cohort. The mortality rate during induction chemotherapy was 10.9%.

Conclusion

This study provides evidence to support the application of the outpatient based, metronomic STIMULus protocol in elderly patients with newly diagnosed and relapsed or refractory AML. The remission rates achieved are comparable to those reported for intensive regimens with potential benefits of reduced toxicity, lower cost, fewer hospital admission and improved quality of life.

062. Evaluating the relevance of NGS-based molecular classification for risk stratification in Australian patients with AML

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Aim

The 2017 European Leukaemia Network (ELN) recommendations for diagnosis and management of acute myeloid leukaemia (AML) in adults recognise mutations in *NPM1*, *FLT3*, *CEBPA*, *RUNX1*, *ASXL1* and *TP53* as having prognostic relevance. In addition, Papaemmanuil *et al* (2016, NEJM) proposed 11 non-overlapping molecular subtypes of AML. Despite accumulating international data, the utility of mutation profiling for AML prognostication has not been directly evaluated among Australian patients. We aimed to evaluate the clinical relevance of mutation analysis for risk stratification in intensively treated Australian patients with AML.

Methods

Cryopreserved bone marrow cells and clinical data were obtained from 74 patients from the Australian Leukaemia Lymphoma Group (ALLG) AML tissue bank and 19 patients treated at Peter MacCallum Cancer Centre (PMCC) with a diagnosis of AML who had undergone remission induction chemotherapy between 2001 and 2015. Sequence analysis was performed using the PMCC 26 gene diagnostic myeloid NGS amplicon panel.

Results

Overall survival (OS) correlated with the favourable, intermediate and adverse risk categories included in the 2010 ELN recommendations ($p=0.0121$) but not with the updated 2017 recommendations ($p=0.0564$). Interestingly, the difference in median OS between *FLT3*-ITD mutated patients with *FLT3*-ITD^{low} (<0.5) or *FLT3*-ITD^{high} (≥ 0.5) allele ratios was not significant in this cohort, and reclassification of *FLT3*-ITD^{low} patients within the 2017 ELN recommendations on this basis did reach statistical significance ($p=0.0283$). Genomic classification of patients determined according to the Papaemmanuil 11-subtype classifier correlated with differences in OS ($p=0.0006$). There was a significant association between the number of mutations detected per patient and decreased OS (median OS for 0, 1-2 and 3-5 mutations was not reached, 1597 and 389 days, respectively, $p=0.0035$). Patients with mutations in chromatin modification/spliceosome genes were identified as having particularly inferior outcomes (median OS 370 days).

063. Exposure-adjusted AEs comparing blinatumomab to SOC chemotherapy in relapsed/refractory B-precursor acute lymphoblastic leukaemia (r/rALL) patients

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Background: Blinatumomab has shown improved overall survival vs SOC in patients with r/r ALL in a randomised phase 3 study. To better evaluate safety, we compared AEs of blinatumomab vs SOC after adjusting for varying treatment exposure times.

Methods: Adults ≥18 years with r/r ALL (refractory, 1st relapse <1 yr, ≥2 relapses or relapse after transplant) were randomised to blinatumomab or SOC (1 of 4 predefined regimens). Blinatumomab was dosed by continuous infusion (4 weeks on/2 weeks off) for up to 5 cycles (9µg/d on d1–7 in cycle 1, then 28µg/d); up to 4 maintenance cycles (4 weeks on/8 weeks off) were allowed for ≤12 months. Exposure-adjusted event rates were calculated as number of events*100/total exposure time.

Results: Median (range) number of cycles were 1 (1–4) for SOC and 2 (1–9) for blinatumomab. The highest exposure-adjusted rates (per 100 patient-years) were for pyrexia (507 SOC vs 376 blinatumomab), anaemia (987 vs 229), thrombocytopenia (750 vs 126) and neutropenia (351 vs 121), all lower in blinatumomab. Febrile neutropenia (365 vs 93) and infections (1216 vs 436) were also both lower in blinatumomab (p<0.0001). Exposure-adjusted rates for neurologic events were 743 SOC vs 472 blinatumomab, with median time (range) to onset of 7 (1–43) d and 7 (1–190) d, respectively, and grade ≥3 cytokine release syndrome (CRS) rates were 0 SOC vs 10 blinatumomab. The most frequent AEs in both cycles 1 and 2 were pyrexia, nausea and anaemia in both arms; CRS events decreased in the blinatumomab arm between cycles 1 and 2 (14% vs 2%). Most fatal AEs were related to infection in both arms.

	SOC N=109 pts		Blinatumomab N=267 pts	
Total exposure, years	14.8		89	
	No. of Events	Exp-Adj Event Rate*	No. of Events	Exp-Adj Event Rate*
All AEs	2037	13764	4108	4616
Gr 3	456	3081	707	794
Gr 4	195	1318	197	221
Fatal	19	128	51	57
Neurologic events	110	743	420	472
CRS	0	0	56	63
All serious AEs	95	642	311	349

*Per 100 patient-years

Conclusions: Blinatumomab showed an AE profile consistent with that previously reported, including similar rates of manageable CRS and neurologic events. Exposure-adjusted AE rates were generally higher in SOC vs blinatumomab, including for cytopenias and infections.

064. CD34+ dose and survival in autograft for relapsed Diffuse large B cell lymphoma.

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Aim

High dose chemotherapy and autologous stem cell transplant (ASCT) remains the standard of care in salvage of chemosensitive relapsed Diffuse large B cell lymphoma (DLBCL). Reports prior to the widespread use of Rituximab indicated survival advantage with higher CD34+ stem cell dose in this setting, however this has not been widely re-examined in the post-Rituximab era. This study aims to assess the effect of CD34+ dose on survival outcomes in patients receiving ASCT for relapsed DLBCL in the last decade.

Method

A retrospective analysis was conducted of patients receiving high dose therapy and ASCT for relapsed DLBCL from March 2006 to July 2016. Cox proportional hazards regression was used to assess associations with overall survival (OS) adjusting for age, revised-international prognostic index (R-IPI), remission status at transplant, prior lines of therapy, CD34+ dose and absolute lymphocyte count (ALC) achieved at days 15 and 30 post ASCT.

Result

Overall, 51 patients received ASCT for relapsed DLBCL with a median age of 61 years at time of transplant. The most significant factors affecting OS across the entire group were prior lines of therapy and status at time of transplant ($p=0.15$ and $p<0.01$ respectively). When patients who achieved complete remission (CR) prior to transplant were analysed alone ($n = 33$): age, R-IPI, CD34+ dose and ALC post ASCT did not impact on OS. In contrast, for patients who were not in CR prior to transplant ($n= 18$), those who received higher CD34+ ($>5 \times 10^9/L$) achieved better OS ($p=0.04$) independent of age and R-IPI. A trend to improved survival was also observed in those who achieved higher ALC at day 15 post-ASCT.

Conclusion

In this study, higher CD34+ doses were associated with improved OS in patients with relapsed DLBCL who did not achieve CR prior to ASCT. Better characterisation of factors contributing to higher CD34+ yield in this group of patients in the future may help to further improve their survival outcomes.

065. Diagnostic utility of endoscopy and biopsy in suspected acute gastrointestinal graft versus host disease (GI-GVHD).

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Background / Aim:

Acute gastrointestinal graft versus host disease (GI-GVHD) is a common and life-threatening complication following haematopoietic progenitor cell transplantation (HPCT). The current diagnostic gold standard is endoscopic biopsy of the gastrointestinal tract, however there are limited data regarding the utility of this approach and the correlation with clinical diagnosis of GI-GVHD.

Methods:

We performed a retrospective cohort study of adults who had undergone endoscopy for suspected acute GI-GVHD within 180 days following allogeneic HPCT for haematological malignancy between 2011-2016. Details included: symptoms at time of referral for endoscopy, type of procedure performed, macroscopic findings on endoscopy, and histological findings following gut biopsy. Correlation was made with clinical GVHD severity scores. Yield of histological GVHD, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and compared for each procedure.

Results:

123 patients were included. Acute GI-GVHD occurred in 59 (48%). The yield of histological GVHD was greater in lower than upper endoscopies (50 vs. 39%). Single upper endoscopy for upper symptoms alone had the lowest yield of GI-GVHD (14%). Combination upper and lower endoscopy demonstrated similar yield of GVHD (50%) and high concordance between histological and macroscopic findings in upper and lower components. The addition of upper endoscopy to lower endoscopy only identified an extra 2 (4%) cases of GVHD. Endoscopy and biopsy findings only identified 75% of those ultimately requiring treatment for acute GI-GVHD.

Conclusion:

Acute GI-GVHD remains a clinical diagnosis supported by available histological evidence. As the current "gold standard" only identifies 75% of clinically treated GI-GVHD, there is a need to refine the current approach and / or develop more robust diagnostic methods. Routine performance of upper endoscopy additional to lower endoscopy for lower gastrointestinal symptoms does not significantly improve the yield or sensitivity for histological GVHD.

066. Assessing Viability and Functionality of Cryopreserved Stem Cells and Outcomes of Hematopoietic Stem Cell Transplantation

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Aim

Success of hematopoietic stem cell transplantation (HSCT) relies on the dose and quality of stem cells being delivered. Cryopreservation results in a decrease in the viability of stem cells, therefore assessment of viability and functionality of post-thaw stem cells is crucial. The current practice is to assess the viability of thawed CD34⁺ cells with a DNA binding dye, such as 7-AAD, which labels dead cells. However, functional markers of viability such as intracellular aldehyde dehydrogenase (ALDH) activity or mitochondrial membrane potential assessment by 1,1',3,3,3'-hexamethylindodicarbo-cyanine iodide [DiIC₁(5)] staining may be a more appropriate means of assessing stem cell engraftment potential. The aim of this study was to assess whether these functional markers of stem cell viability [ALDH and DiIC₁(5)] are predictive of engraftment outcomes.

Method

Eighty autologous hematopoietic stem cell transplants performed at Royal North Shore Hospital, Sydney between 2002 and 2014 were assessed. The dose of viable CD34⁺ cells delivered to each patient was determined using the current practice of viability assessment (7AAD exclusion) as well as the assay using DiIC₁(5) staining and ALDH activity. Results from these assays were correlated with post-transplant outcomes, which include time to engraftment, durability of engraftment and survival endpoints.

Results

In this patient group, the doses of CD34⁺/DiIC₁(5)⁺ and ALDH⁺/DiIC₁(5)⁺ delivered correlated with time to neutrophil engraftment ($P < 0.05$). There was a strong association between time to neutrophil engraftment and the number of prior lines of therapy received. No significant correlation between the numbers of infused CD34⁺/DiIC₁(5)⁺, ALDH⁺/DiIC₁(5)⁺ or viable CD34⁺/ALDH⁺ cells and platelet engraftment was observed. Time to neutrophil engraftment was predictive of disease-free survival (DFS) and overall survival.

Conclusion

These data suggest that assessing viability of stem cells by using assays for DiIC₁(5) and ALDH may prove useful in predicting time to neutrophil engraftment after autologous transplantation.

067. Donor haemoglobin before marrow harvest is associated with stem cell yield and post-harvest haemoglobin change

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Aim

Assess the effect of low donor haemoglobin on bone marrow harvest outcomes

Method

Retrospective review of marrow harvests performed at Westmead Hospital (2006-2017). Gender specific institutional reference ranges were applied to FBC. Cells were enumerated using automated cell counting and flow cytometry for CD34 and CD3 by ISHAGE gating. Paired t-test, Chi-square and Pearson correlation were used.

Results

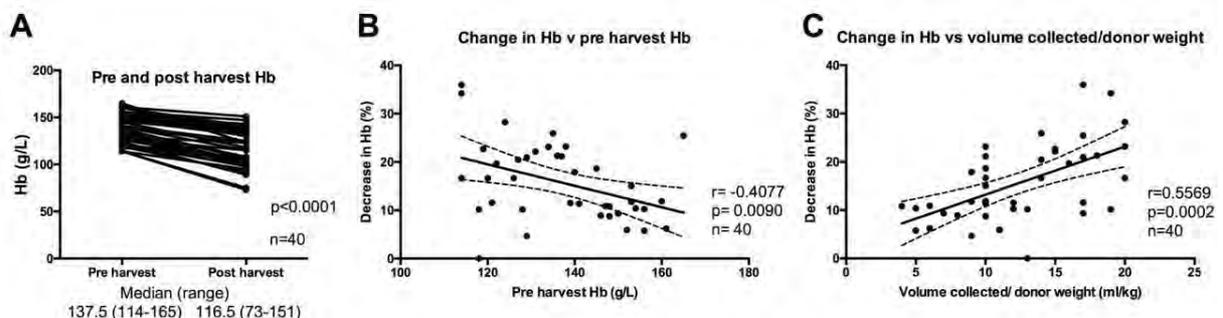
80 donors were identified. FBC were assessed a median of 1 day pre (range 0-21 days) and 0 days post-harvest, both assessed in 40 donors. Pre-harvest Hb, WBC, platelet count or MCV were outside of the reference range in 22 (27.5%) with anaemia (median Hb in anaemic donors 118g/L, range 102-129) the most frequent abnormality (n= 10, 12.5%).

In 15/80 and 2/80, $<2 \times 10^6$ CD34+ cells and $<2 \times 10^8$ total nucleated cells (TNC) per kg of recipient weight was harvested, respectively. Donors were grouped into quartiles (n=20 per quartile) based on pre harvest Hb, collected CD34 and TNC per kg recipient was lower in donors from the lowest Hb quartile compared to the remainder (median CD34 2.3 v 3.3×10^6 /kg, $p=0.025$ and median TNC 3.4 v 4.0×10^8 /kg, $p=0.032$). Higher TNC per kg recipient weight was associated with male donors ($p=0.011$). Recipients undergoing transplant outside Westmead Hospital had significantly higher CD34+ and TNC counts per kg recipient weight likely due to excess of paediatric recipients at outside institutions (median recipient weight 55 v 77 kg, $p=0.001$).

Decrease in post-harvest Hb (median 19g/L) (Fig 1A) was associated with lower pre collection Hb (Fig 1B), higher collection volume (Fig 1C), low donor weight ($r=-0.398$, $p=0.0112$) and female gender (median change in Hb 20% v 11% for males, $p=0.007$). Three (3.8%) required post-harvest transfusion.

Conclusion

Lower pre-harvest Hb was associated with a reduction in stem cell yield and a greater fall in Hb after harvest.



068. A single-institution, retrospective comparison of BEAM and BuMel as conditioning before auto-HCT in NHL patients

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Background, aim and method

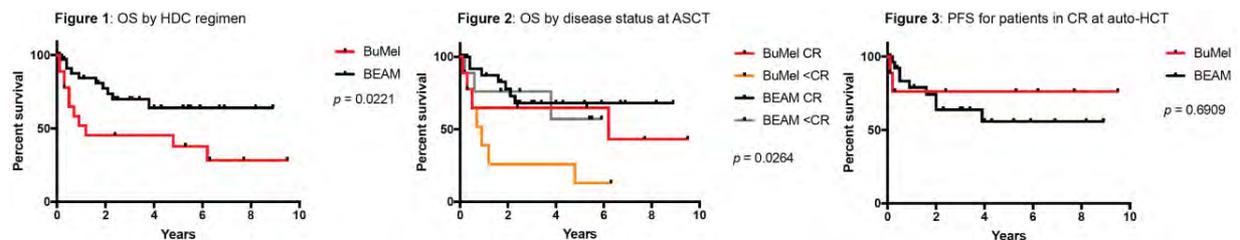
The optimal high-dose chemotherapy (HDC) regimen in patients undergoing auto-HCT for relapsed/refractory non-Hodgkin lymphoma (NHL) is unknown. The most commonly used is BEAM (carmustine, etoposide, cytarabine and melphalan). Three small retrospective series have examined the efficacy of HDC with busulfan and melphalan (BuMel) in patients with NHL. No study has directly compared the outcomes of BuMel and BEAM HDC in patients with NHL.

We performed a retrospective, single-centre review comparing the outcomes of NHL patients undergoing auto-HCT with BuMel or BEAM conditioning between 2007 and 2017, focusing on overall survival (OS), progression-free survival (PFS), treatment-related mortality (TRM), use of total parenteral nutrition (TPN) and engraftment.

Results

38 patients received BEAM and 19 BuMel (including 17 patients with NHL). Median follow-up was 23 months. Median time to neutrophil engraftment was significantly shorter for BEAM patients (9 vs. 11 days; $p = 0.0002$), while there were no differences in platelet engraftment, days of hospitalisation or use of TPN.

Five-year OS was 64% for BEAM patients and 38% for BuMel patients ($p = 0.02$; **Figure 1**). 82% of deaths in BuMel patients were due to disease. 58% of patients in the BuMel group died, compared with 26% in the BEAM group ($p = 0.04$). TRM was 5% in the BuMel group (one death) and zero in the BEAM group ($p = \text{NS}$). There was no significant difference in PFS.



Survival was particularly poor in BuMel patients in less than CR at auto-HCT (five-year OS 13%, $p = 0.03$; **Figure 2**). In contrast, BuMel patients in CR at auto-HCT had favourable five-year PFS (76%; **Figure 3**), with the impression (despite small numbers) of a plateau in the curve.

Conclusion

BEAM patients had significantly superior OS to BuMel patients, largely due to early disease-related deaths in BuMel patients in less than CR at the time of auto-HCT. We question whether there is any benefit of offering BuMel auto-HCT to these patients. In contrast, BuMel patients in CR at the time of auto-HCT had favourable long-term PFS. We believe that this is the largest reported series of patients with NHL undergoing auto-HCT with BuMel conditioning

069. Late relapse in Acute Myeloid Leukemia post allogeneic stem cell transplantation

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Aim

The study was performed to the survival outcomes post allogeneic stem cell transplantation for AML from 2010 to 2015 in all patients within the national ABMTRR database and Western Australian AML cohort.

Method

An analysis was undertaken of all consecutive adult (over age of 16years) patients transplanted in Australia from 2010 to 2015, data obtained from the ABMTRR in patients over the. Relapse rates in AML post allogeneic transplantation in Western Australian (n=107, from 2005 to 2015) was studied. We used Kaplan-Meier method to assess overall survival (OS), log rank to assess significance of differences with p value <0.05 considered statistically significant (SPSS V24).

Result

Data from the ABMTRR showed that the 10 year survival probability of HLA identical sibling donor HCT for AML recipients was 53%, 38% and 19% in patients transplanted in CR1 (n=519), CR2 (n=166) and all other (n=173). Similarly for survival probability for matched unrelated allografts performed in CR1 (n=346), CR2 (n=240) and all other (n=149) was 51%, 46% and 23% respectively. In the Western Australian cohort, early relapse within 6 months, occurred in 11 of 107 patients, 19 patients relapsed between 6 months and 3 years. Very late relapses (more than 3 years) occurred in five patients transplanted in CR2 (4.67%) in 56.13 months (range 41.92-98.10). Extramedullary AML was seen in 8 out of 23 patients of the late relapses (34.78%).

Conclusion

Overall survival is higher in patients with AML transplanted in CR1 irrespective of either matched related or unrelated donors. The aims and challenges in identifying suitable patients for early transplantation in CR1 if suitable donor is available are noted. Late relapse post-AML relapse is uncommon and possible treatment strategies of salvage chemotherapy, DLI and second allografts are discussed.

070. Long-term efficacy and safety of the RESONATE study: ibrutinib in previously treated chronic lymphocytic leukemia

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Aims: Ibrutinib, a first-in-class, once-daily inhibitor of Bruton's tyrosine kinase, is FDA-approved for all patients with CLL/SLL. We report updated safety and efficacy up to 4 years from the phase 3 RESONATE™ trial.

Methods: Patients had ≥1 prior therapy. Patients received 420 mg ibrutinib PO until PD or ofatumumab up to 24 weeks. At interim analysis, the DMC declared superiority of ibrutinib vs ofatumumab; ibrutinib access was recommended for all ofatumumab patients. Ofatumumab patients were censored at crossover for OS.

Results: 391 patients were randomized to receive ibrutinib (n=195) or ofatumumab (n=196). Median age was 67 years; 57% had Rai stage III/IV. With median follow-up of 44 months (53 months max) for ibrutinib arm, PFS was significantly longer for ibrutinib vs ofatumumab (median NR vs 8 months, [HR 0.133; $P < 0.0001$]) with significant benefit across subgroups. PFS with ibrutinib for del11q subgroup trended to have the most favorable outcome but was not statistically different between patients with del17p or del11q or without these FISH abnormalities. At analysis, with 68% of ofatumumab patients crossing over to ibrutinib, OS was longer for ibrutinib vs ofatumumab (median OS NR for either arm). ORR for ibrutinib was 91%; CR/CRi rates (now 9%) increased over time. Baseline cytopenias improved with extended ibrutinib therapy. AE profile of ibrutinib was consistent with previous reports. Major hemorrhage, Gr ≥3 atrial fibrillation, and Gr ≥3 hypertension occurred in 6%, 6%, and 8% of patients, respectively. Incidence of most Gr ≥3 AEs decreased 1 vs year 2-3: neutropenia: 18% vs 8%; pneumonia: 11% vs 4%; atrial fibrillation: 4% vs 2%, respectively. Discontinuations were most frequently PD (27%) and AE (12%). At analysis, 90 ibrutinib patients (46%) continue ibrutinib on study.

Conclusions: Long-term treatment with ibrutinib in this international phase 3 RESONATE study is tolerable and shows sustained PFS and OS.

071. Outcomes of standard-of-care regimens in treatment-naïve CLL patients with unmutated immunoglobulin heavy chain variable genes

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Aim

When treated with traditional chemoimmunotherapy regimens, patients with unmutated immunoglobulin heavy chain variable (IGHV) display worse clinical outcomes than those with mutated IGHV. Ibrutinib, a first-in-class covalent Bruton's tyrosine kinase inhibitor, significantly improved progression-free survival (PFS; hazard ratio [HR], 0.16; 95% CI, 0.09-0.28; $p < 0.001$) and overall survival (HR, 0.16; 95% CI, 0.05-0.59; $p = 0.001$) versus chlorambucil, with similar outcomes between IGHV mutated and unmutated patients (Burger, et al. *NEJM* 2015). An unadjusted naïve comparison was performed between ibrutinib, bendamustine and rituximab (BR) and fludarabine, cyclophosphamide, and rituximab (FCR) treatment-naïve CLL patients.

Method

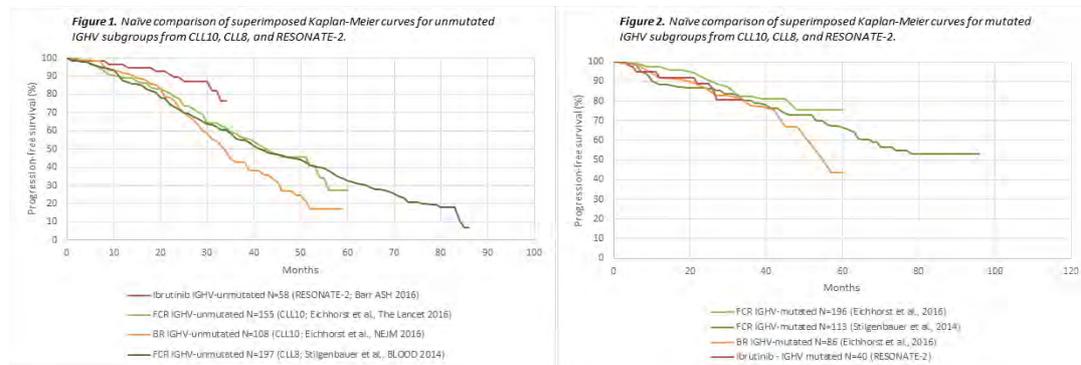
Baseline characteristics in the CLL8, CLL10, and RESONATE-2 trials were evaluated. An unadjusted naïve comparison of PFS using published Kaplan-Meier curves of mutated and unmutated IGHV subpopulations treated with FCR, BR, or ibrutinib was performed. Digitized curves provided estimates of 30 month PFS rates.

Result

48% of ibrutinib-treated patients in RESONATE-2, 55% and 68% in the FCR and BR arms of CLL10, and 63% in the FCR arm of CLL8 had unmutated IGHV; proportion of male gender: 88%, 71%, 74%, and 74%, respectively; RESONATE-2 patients were older (median age: 73, 62, 61, and 61 years, respectively). RESONATE-2 and CLL10 excluded patients carrying del17p, but they represented 10% in CLL8. In patients with unmutated IGHV: ibrutinib-treated patients appear to maintain PFS longer than FCR- or BR-treated patients; estimated difference in PFS at 30 months was >20% (Figures 1-2). Estimated 30-month PFS rates for patients with unmutated IGHV were 87% for ibrutinib-treated patients in RESONATE-2, 64% for the FCR arm of CLL8, and 65% and 59% for the FCR and BR arms of CLL10.

Conclusion

Patients in RESONATE-2 were >10 years older, patients with del17p were excluded from CLL10 and RESONATE-2. Unmutated IGHV in ibrutinib-treated CLL patients is not a prognostic factor for adverse outcomes, as is seen with chemoimmunotherapy.



072. Analysis of Overall Survival of Idelalisib with Bendamustine & Rituximab in Patients with Relapsed/Refractory CLL

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Introduction: We have previously reported that idelalisib (IDELA), a selective PI3K-delta inhibitor, administered in combination with bendamustine/rituximab (BR) improves progression-free survival (PFS) compared with BR alone after a median follow-up of 12 months. This study was unblinded by the independent data monitoring committee at first interim analysis for efficacy. We now present updated data on overall survival (OS).

Methods: 416 patients (pts) with relapsed/relapsed (RR) CLL were enrolled. PFS was the primary endpoint, with OS a secondary endpoint. Patients were randomized to BR for 6 cycles Q28 days (B=70mg/m² D1, D2 of each cycle; R=375mg/m² C1 and 500mg/m² C2-6) and IDELA 150mg BID or placebo (administered until IRC-confirmed PD), death, intolerable toxicity or withdrawal of consent. .

Results: The ITT population: 42% ≥65 years; Rai stage III/IV 46%; high-risk features (del[17p]/p53mut 32.9%, unmutated IGHV 83.2%); median number of prior therapies: 2 (range 1–13); and median follow-up 21 months. Overall by ITT and IRC, 260/416 pts (IDELA/placebo 95/165) have met the primary endpoint of PD/death. Median OS (mo) of IDELA+BR vs BR+placebo was not reached vs 41 (HR=0.67; p value 0.036; 95% CI 0.47, 0.96). Safety findings were similar to previously reported: Serious AEs occurred in 147 (71%)/94 (5%) IDELA/placebo arms, respectively. Commonly occurring SAEs were infections, infestations, febrile neutropenia and pneumonia. Opportunistic infections (*Pneumocystis jirovecii* pneumonia [PJP]/cytomegalovirus [CMV]) in the IDELA arm was 5/13 vs 0/3 in the placebo arm.

Conclusion: IDELA in combination with BR is superior to BR alone with regard to OS in RR CLL. The improvement in OS was observed across risk categories. Opportunistic infections and SAEs were more frequent in the IDELA vs placebo arm. Results of IDELA-containing regimens may be further improved with implementation of PJP prophylaxis and CMV monitoring measures. This regimen represents an important new option for pts with RR CLL. https://ash.confex.com/data/abstract/ash/2016/0/6/Paper_91760_abstract_210473_0.png

073. Waldenstroms macroglobulinaemia is immunologically distinct from Myeloma with exhausted clonal T cells expressing LAG3

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Background: T cell directed therapies are showing promise in haematological malignancies. In Multiple Myeloma (MM), PD-1 inhibition was only effective in combination with Imids[®]. This may be due to broad inhibition of anti-tumour immune responses in MM. We have described telomere-independent senescence in clonally expanded T cells in MM along with increased MDSC, increased Treg/Th17 balance and the induction of Treg through trogocytosis. Less is known about the immune system in Waldenstroms macroglobulinaemia (WM) and we hypothesised that greater understanding of the immune response may inform immunotherapy.

Methods: Blood was collected from patients with informed consent, peripheral blood mononuclear cells were prepared and analysed by flow cytometry to determine subset number and phenotype. T cells clones were analysed using the TCRV β IOTest Beta Mark kit.

Results: Clonal cytotoxic T-cells are present in the blood of 70% of WM (n=20), and 48% of MM (n=120) rising to 70% with Imid[®] exposure. In WM (n=12) there was a normal Treg/Th17 ratio whilst in MM it was significantly increased (n=32; t=4.08, p<0002). WM patients have a normal percentage of Treg cells in the blood, and an absence of trogocytosis which in MM induces an average of 12% of T cells to become Treg. MDSC are highly elevated in MM (mean (106x10⁷/L) but not in WM (0-3x10⁷/L). T cell clones exhibit telomere-independent senescence in MM, however in WM they have an exhausted phenotype with low levels of PD-1 but increased LAG3 expression. We are exploring the functional effects of LAG3 interacting with its ligand HLA-DR, which is expressed by malignant cells.

Conclusion: Multiple immunological differences exist between WM and MM which suggest that the immune microenvironment in WM is better suited to T cell directed therapies, and that LAG3 inhibition is a potential therapeutic target.

074. Prognostic impact of PET-CT after first-line immunochemotherapy for follicular lymphoma in the Phase-III GALLIUM study

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Aim: We evaluated the prognostic impact of PET complete metabolic response (CMR) status for the large FL patient cohort enrolled in the Phase III GALLIUM study (NCT01332968).

Method: 1202 pts with previously untreated FL were randomised to induction therapy comprising one of 3 pre-selected chemotherapy regimens (CHOP, CVP, or Bendamustine) plus 1000mg obinutuzumab (G; D1, 8, 15 C1 then D1 subsequent cycles) or 375mg/m² rituximab (R; D1 each cycle). Baseline and end of induction (EOI) PET scans were assessed by an independent review committee (IRC), applying the 2007 International Harmonisation Project (IHP) criteria and 2014 Lugano Criteria.

Result: Of 609 pts with a baseline PET, 595 had detectable lesions. 543 had an evaluable PET (IHP 2007) and 519 with paired baseline and EOI PETs (Lugano 2014). Pts with unresponsive or progressive disease prior to EOI were excluded from landmark PFS analyses. At EOI 390/595 (65.5%) pts achieved CMR using IHP 2007 and 455/595 (76.5%) using Lugano 2014 criteria. After a median follow-up of 41 months, EOI CMR status, while highly predictive of PFS using both criteria, had the greatest prognostic impact using Lugano 2014 criteria: CMR vs non CMR: HR 0.22; (95% CI 0.13–0.34; p<0.0001), and OS HR 0.22; (95% CI 0.11 0.45; p<0.0001). 2.5-year PFS was 87.4% (95% CI 83.8-90.3) for CMR pts compared with 54.1% (95% CI 39.5–66.5) non-CMR: OS was 96.6% (95% CI 94.4–97.9) vs 83.8% (95% CI 72.6–90.7). IRC PET status retained prognostic value in both G- and R-treated populations.

Conclusion: This large prospective analysis confirms that PET status after 1L immunochemotherapy for FL, applying the current Lugano response 2014 criteria, is an early prognostic factor for both PFS and OS. A 13% absolute difference in OS at only 2.5 years after induction is highly significant in this usually indolent lymphoma and establishes EOI PET as the gold-standard imaging modality of response assessment in FL.

075. A retrospective pharmaceutical financial benefits analysis of clinical trial participation

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Aim

Clinical trial participants receiving investigational new agents which subsequently become registered for their trialed indication effectively receive free early access to efficacious treatment. The purpose of this study is to calculate both the financial benefit of such access, as well as pharmaceutical cost avoidance through free access to Pharmaceutical Benefit Scheme (PBS) listed agents through clinical trial participation at a single Haematology Clinical Research Unit (HCRU).

Method

All recruiting clinical trials between 1 January 2006 and 31 December 2016 performed at the HCRU, Concord Repatriation General Hospital (CRGH), Sydney were reviewed. Investigational new agents which subsequently became approved in a medicines regulatory authority for their trialed indication were identified, as well as any subsequent Therapeutic Goods Administration (TGA) approval and PBS listings. Individual patient doses were determined from pharmacy dispensing records. Financial benefit and cost avoidance from PBS savings were calculated based on the PBS pricing lists, or where these are not PBS listed, from UpToDate[®].

Results

A total of 134 studies were conducted in the HCRU during the period 1 January 2006 to 31 December 2016. Out of these, 34 clinical trials involving a total of 203 patients yielded a financial benefit or cost avoidance. We estimated \$14,513,890 in financial benefit and \$3,189,899 in cost avoidance, with a total value of \$17,703,789. Of note, 91% of this total was derived in the last five years of this study period.

Conclusion

Cost avoidance from sponsored clinical trials results ultimately in substantial savings in health costs. Investigational new agents that become listed for their trialed indication represent significant value to patients, hospitals and the Australian PBS. Financial benefit is an under-recognised benefit of conducting clinical trials. Our study shows substantial financial benefit of clinical trial participation, and may represent greater value than cost avoidance.

076. Efficacy and safety of pomalidomide (POM) + low-dose dexamethasone (LoDEX) after second-line lenalidomide (LEN)-based treatment

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Aim: We present updated safety and efficacy results only from cohort A of the MM-014 phase 2 trial, in which patients with relapsed or refractory multiple myeloma (RRMM) received POM+LoDEX immediately after relapsing or being refractory to second-line LEN-based therapy. The study was amended to include cohort B (POM+LoDEX+daratumumab).

Methods: Adult patients with MM, 2 prior lines of therapy, and progressive disease after ≥ 2 cycles of second-line LEN-based treatment received POM+LoDEX. Overall response rate (ORR) by modified IMWG criteria was the primary endpoint. Key secondary endpoints included time to response (TTR), progression-free survival (PFS), second primary malignancies (SPMs), and biomarkers.

Results: Of 51 enrolled patients, 76.5% discontinued treatment. Most patients (88.2%) were refractory to their last LEN treatment (median treatment duration 24.6 mos) and 72.5% had prior bortezomib.

ORR was 29.4% (2.0% complete response, 9.8% very good partial response, and 17.6% partial response [PR]); median follow-up was 13.6 mos. Median TTR was 1.9 months; 66% of patients had ongoing response at 1 year, and 15.7% achieved minimal response (MR). Median PFS was 13.8 months. Anemia and infections were the most common grade 3/4 adverse events (AEs). Table presents additional results.

CD3⁺ and CD3⁺/CD8⁺ T-cell populations increased significantly from baseline to after treatment (67.8% vs 72.6% and 32.1% vs 36.9%, respectively; $P < .05$). Mean relative changes in CD3⁺ and CD3⁺/CD4⁺ T-cell populations from baseline were significantly greater in patients with response vs patients with no response (10.4 vs -0.8 and 4.2 vs -3.5; $P < .05$).

Conclusions: Results confirm the safety and efficacy of POM+LoDEX following second-line LEN-based treatment failure in patients with RRMM. Hematologic AE rates improved and median PFS was longer compared with previous reports of POM+LoDEX use in later treatment lines. In addition, patients who achieved \geq MR had similar PFS rates as those who achieved \geq PR.

Grade 3/4 adverse events, %	N = 51
Anemia	25.5
Neutropenia	11.8
Infections	19.6
Pneumonia	9.8
SPMs, %	0
2-Year PFS, %	
Intent to treat	48.6

≥ PR	69.1 ^a
≥ MR	69.4 ^a
POM treatment duration by response, months	
≥ PR (n = 15)	11.5
≥ MR (n = 23)	10.5
MR (n = 8)	7.7
Stable disease (n = 21)	3.7

^a Subject to survival biases.

077. Survival Outcome for Newly Diagnosed Multiple Myeloma Over Last 20 Years in New Zealand

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Aim

The aim of this study is to evaluate the overall survival of patients with newly diagnosed multiple myeloma over the last 20 years in New Zealand, including those older than the age of 70.

Method

All new cases of MM diagnosed between 1 January 1995 and 31 December 2014 were extracted from the New Zealand national cancer registry and analysed. Overall survival (OS) was calculated from the time of diagnosis to the time of death. Patients who were not deceased at the time of data extraction were assumed alive and censored at that point. All statistical analyses were performed using SPSS version 20, and a p-value of less than 0.05 was deemed significant.

Result

A total of 5096 new cases of multiple myeloma were diagnosed during this period. The number of new cases rose from 208 in 1995 to 372 in 2014, and the incidence increasing from 5.66 to 8.25 per 100,000 during the same period. The OS for patients diagnosed between 1995-1999, 2000-2004, 2005-2009, and 2010-2014 were 20.0 months, 27.5 months, 37.0 months and 46.7 months, respectively ($p < 0.001$). Patients aged between 70-79 experienced the greatest improvement in OS with median survival increased from 17.8 to 42.8 months ($p < 0.001$). During the same period, patient aged 80 or above did not experience any significant improvement in OS (median OS changed from 9.1 to 11.7 months, $p = 0.216$). On multivariable analysis, age (HR 1.051), gender (HR 1.125 for males), and socio-economical deprivation (HR 1.022) were found to be independent prognostic factors for survival.

Conclusion

The long-term survival outcome for patients with MM has improved in recent years, but age continues to be a significant independent negative prognostic factor for survival.

078. Overall survival with carfilzomib and dexamethasone versus bortezomib and dexamethasone in the ENDEAVOR trial

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Background: In the ENDEAVOR trial, progression-free survival (PFS) was shown to be significantly longer with carfilzomib (K) and dexamethasone (d) than with bortezomib (V) and dexamethasone (median 18.7 months vs 9.4 months, hazard ratio [HR], 0.53; 95% confidence interval [CI], 0.44–0.65; $P < 0.0001$; Dimopoulos et al. *Lancet Oncol.* 2016). Results from a planned second interim overall survival (OS) analysis of ENDEAVOR are presented.

Methods: Study design has been previously reported (Dimopoulos et al. *Lancet Oncol* 2016). OS was compared between treatment arms using a stratified log-rank test.

Results: The median treatment duration was 48 weeks for carfilzomib (N=464) and 27 weeks for bortezomib (N=465), with a median follow up of 38 months for Kd and 37 months for Vd. Efficacy results are shown in the table. The overall survival benefit was consistent regardless of prior V therapy, age, baseline ECOG PS, cytogenetic risk status and prior number of therapies (Table).

	Kd (N=464)	Vd (N=465)
Median OS (95% CI), months	47.6 (42.5-NE)	40.0 (32.6-42.3)
All-cause mortality, HR (95% CI)	0.791 (0.648-0.964); 1-sided p=0.01	
OS by prior V therapy, HR		
Yes	0.84	
No	0.75	
OS by age subgroup, HR		
<65 years	0.85	
65-74 years	0.71	
>75 years	0.84	
OS by baseline ECOG PS, HR		
0	0.81	
1	0.80	
2	0.50	
OS by cytogenetic risk status, HR		
High risk	0.83	
Standard risk	0.85	
OS by number of prior lines of therapy		
1	0.83	
2-3	0.76	

The most frequent any-grade adverse events in the Kd arm were (Kd vs Vd) anemia (42.5% vs 28.3%), diarrhea (36.3% vs 40.6%), pyrexia (32.4% vs 15.4%), dyspnea (32.2% vs 13.6%), fatigue (32.2% vs 30.7%), and hypertension (32.2% vs 9.9%). Grade 3 or higher adverse events were experienced by 81.4% of patients in the Kd arm and 71.1% of patients in the Vd arm.

Conclusions: ENDEAVOR was the first randomized phase 3 trial to directly compare two different PIs in relapsed/refractory multiple myeloma. Kd patients had significantly longer OS compared with Vd patients. Safety results were comparable with those previously reported in the PFS interim analysis for ENDEAVOR.

079. Pomalidomide versus pomalidomide, dexamethasone maintenance following pomalidomide, dexamethasone induction in relapsed/refractory myeloma (ALLG MM14)

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Aims

To evaluate the effect of maintenance with pomalidomide (POM) alone (Arm 1) versus POM-low dose dexamethasone (LoDEX) (Arm 2) on survival (PFS and OS) and response (overall response rate (ORR)) in relapsed myeloma patients refractory to lenalidomide (R-LEN) demonstrating at least stable disease (SD) following salvage with POM-LoDEX induction.

Methods

Multicentre, open-label, randomised phase 2 study of relapsed R-LEN patients (≥2 prior therapies). POM 4mg d1-21/28d cycle was administered alone (Arm 1) or with LoDEX (40mg weekly) (Arm 2) as maintenance following induction: 4 cycles of POM-LoDEX. Treatment continued until toxicity/progression.

Results

154 patients from 11 sites were enrolled (M:F 80:74), median age: 67 years (35-88). Median prior treatment lines: 4.5 (2-14). All were lenalidomide refractory, 127 (82.5%) were also bortezomib refractory (double refractory). 72 (47%) patients achieved ≥SD with POM-LoDEX induction and were randomised: Arm 1=35, Arm 2=37. After a median follow-up of 19m, median PFS for all patients from study entry was 3.9m (IQR 2.1–8.1m). PFS for randomised patients (from time of randomisation) was 2.5m (Arm 1) versus 5.6m (Arm 2) (p=0.108). The PFS hazard rate for Arm 2 was relatively constant compared to Arm 1, which was initially double that of Arm 2 but by 9 months was similar to Arm 2 (Figure 1.), suggesting that the initial apparent advantage of POM-LoDEX maintenance is not sustained. Median OS: 13.2m (IQR 6.3–26.8m). For randomised patients, median OS was 25m (Arm 1) versus 14.5m (Arm 2) (p=0.33) (Figure 2.) ORR for all patients was 37% [CR=3 (2%), VGPR=11 (7%), PR=43 (28%)].

Conclusion

In patients with relapsed myeloma, after initial disease control/debulking with POM-LoDEX induction, POM maintenance may be as effective at sustaining disease control as continuation of POM-LoDEX (if not more effective). Correlative studies are underway to investigate the immunological mechanisms behind this observation.

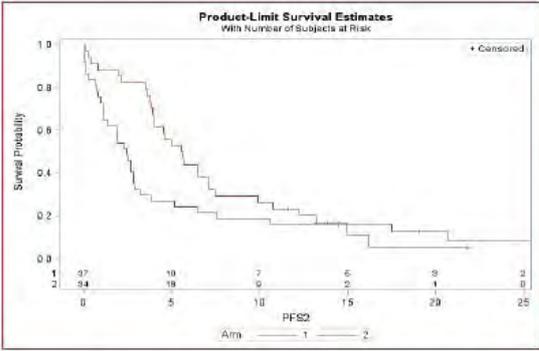


Figure 1. PFS for randomised patients (m)

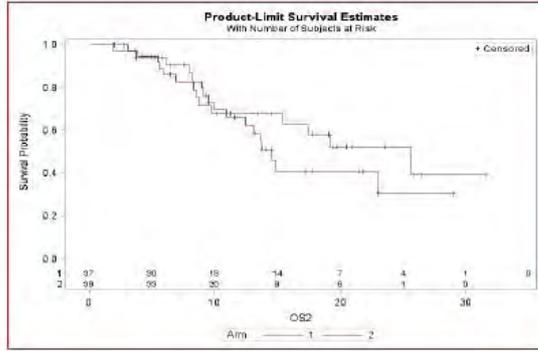


Figure 2. OS for randomised patients(m)

080. Renal impairment in myeloma – characteristics, treatment and outcomes, Australia & New Zealand Myeloma Registry

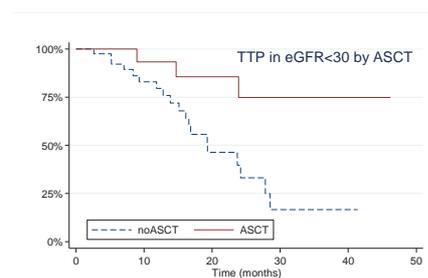
Ho P¹, Moore E², McQuilten Z², Bergin K³, Augustson B⁴, Blacklock H⁵, Horvath N⁶, King T¹, McNeil J², Mollee P⁷, Quach H⁸, Reid C², Rosengarten B⁹, Walker P¹⁰, Wood E², Spencer A³
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Aims & Methods: Renal impairment (RI) is a poor prognostic factor in multiple myeloma (MM). Data from newly diagnosed MM patients from Feb 2013 to Mar 2017 in the Australian and New Zealand Myeloma Registry were analysed to assess characteristics, treatment and outcomes of patients with RI.

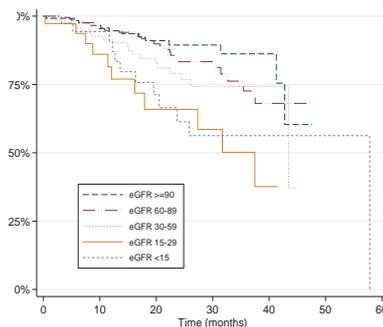
Results: 843 patients had eGFR available at diagnosis: 36% had RI (eGFR < 60 ml/min) (23% 30-60 ml/min; 6% 15-30 ml/min; 7% <15 ml/min). Mean age of RI patients was higher (72 vs 64 yrs), and advanced stage (ISS III) was more prevalent (64% vs 12%, p<0.001). High risk FISH features/high LDH (55% vs 47%, p=0.03), anaemia (46% vs 14 %, p<0.001) and diabetes mellitus (13% vs 7%, p=0.01) were more prevalent in RI but bone lesions were less prevalent (53% vs 65% p=0.001). Bortezomib was given less frequently at diagnosis in RI (83% vs 91%, p=0.004). While response rates (≥PR) were similar in patients with RI (83% vs 85%, p=0.54), TTP & OS decreased with reduction in eGFR. Fewer RI patients ≤70y received ASCT (60% vs 73%, p<0.03), although it was performed at all levels of renal function. In RI patients, those who received ASCT had longer TTP (HR 0.41, 95%CI 0.17-0.96, p=0.04) & OS (HR 0.30, 95%CI 0.08-1.05 p=0.06) compared with no ASCT. This included severe RI (<30 ml/min), with longer TTP (HR 0.21, 95%CI 0.05-0.86, p=0.03) & OS (HR 0.10, 95%CI 0.01-0.82, p=0.03). There was no difference in TTP (HR 1.06, 95%CI 0.57-1.97, p=0.86) or OS (HR 0.89, 95%CI 0.29-2.79, p=0.85) between patients with & without RI who had ASCT.

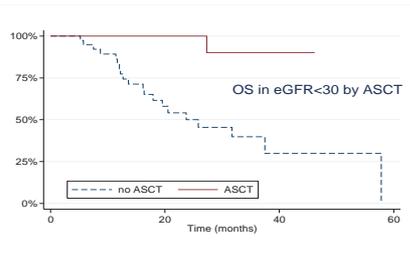
Conclusions: RI occurred in one-third of newly diagnosed MM, with higher prevalence of advanced stage & high risk features, and shorter OS. ASCT was performed in 60% of RI patients ≤70y and was associated with superior TTP and OS, including in those with severe RI.

(a) TTP & OS with & without ASCT for GFR <30 ml/min



(b) OS by renal function grouping





081. Peripheral neuropathy in relapsed/ refractory myeloma treated with carfilzomib vs comparators in Phase 3 trials

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Aims

Peripheral Neuropathy (PN) is a dose-limiting toxicity for some anti-multiple myeloma (MM) agents. Carfilzomib (K) was evaluated in 2 recent phase 3 studies in relapsed/refractory MM (RRMM) patients.

Methods

This analysis evaluated PN rates in ASPIRE (K [27 mg/m²]-lenalidomide [R]-dexamethasone[d] [KRd] vs Rd in relapsed MM) and ENDEAVOR (Kd [K 56 mg/m²] vs bortezomib [V]-d in RRMM). We evaluated grade ≥ 2 PN during treatment, patient-reported outcomes (LS mean difference QLQ-C30 pain, FACT/GOG-neurotoxicity subscales), and progression-free survival (PFS) in patients with baseline history of PN.

Results

In ASPIRE, grade ≥ 2 PN rate was low (8.9% [KRd] vs 8.0% [Rd]; OR [95% CI]: 1.132 [0.683, 1.876]; p=0.685). Pain subscale scores were similar between arms (ITT [95% CI]: -1.02 [-3.77, 1.73]; p=0.47). Median PFS was similar for K patients with baseline grade ≥ 2 PN vs any baseline PN (24.2 vs 23.2 months). In ENDEAVOR, grade ≥ 2 PN rate during the study was significantly lower with Kd vs Vd (6.0% vs 32.0%; OR (95% CI) 0.137 (0.089, 0.210); p<0.0001). Patients had significantly lower pain (ITT [95% CI] -2.35 [-4.30, -0.39]; p=0.0186) and neurotoxicity subscale (Safety, [95% CI] 0.84 [0.40, 1.28]; p=0.0002) scores with Kd vs Vd. Median PFS (months) with Kd vs Vd in patients with BL history of grade ≥ 2 PN was 18.6 vs 5.6; HR [95% CI] 0.42 [0.266, 0.677], and 18.7 vs 9.4; HR [95% CI] 0.54 [0.410, 0.715] in patients with any baseline PN.

Conclusion

In ENDEAVOR, Kd resulted in less PN vs Vd; in ASPIRE, PN rate was similar for KRd vs Rd. Median PFS was longer with KRd and Kd vs Rd and Vd, respectively, in patients with BL grade ≥ 2 PN. Improved pain and neurotoxicity outcomes with K may be attributed to better disease control and/or lower PN rates.

082. Tolerance induction in graft versus host disease: The role of interleukin-2

Koreth J

CD4+CD25+FOXP3+ regulatory T cells (Treg) are critical for the maintenance of immunologic tolerance. Although Treg comprise only ~5-10% of circulating CD4+ T-cells, they can dominantly suppress autoreactivity and control innate and adaptive immune responses. Treg impairment is associated with loss of tolerance, autoimmunity, and chronic Graft-versus-host disease (GVHD). Adoptive Treg transfer can ameliorate GVHD in preclinical models, but GMP-grade *ex-vivo* Treg expansion remains challenging.

Low-dose Interleukin-2 (IL-2) therapy is an alternative strategy to augment Treg *in-vivo*. At physiologic concentrations IL-2 is critical for the development, expansion, activity and survival of Treg. In a phase 1 trial in steroid-refractory chronic GVHD, once-daily subcutaneously administered IL-2 (1×10^6 IU/m²) was safe, and preferentially enhanced CD4+ Treg *in-vivo*, with clinical responses in approximately half of the treated patients. A phase 2 trial confirmed and extended these findings with regards both its clinical and immunologic effects. The talk will review Treg homeostatic dysregulation that underlies chronic GVHD, summarize the clinical and immunologic effects of low-dose IL-2 therapy, and highlight efforts to better define its role in chronic GVHD treatment.

083. Long-term outcomes of BMT - factors determining survivorship

Kerridge I

Royal North Shore Hospital

Abstract not supplied

088. Real-world experience of newly diagnosed myeloma patients: the Canadian Experience

Reece D

Princess Margaret Cancer Centre / University of Toronto

The Myeloma Database developed by the Princess Margaret Cancer Centre Myeloma Program contains demographic and treatment information on approximately 4000 patients. One use of this database has been to analyse the outcomes of different subsets of myeloma patients to ensure that certain benchmark results are achieved in a socialized medicine environment with restricted funding of novel agents.

Beginning in 2008, our newly diagnosed myeloma patients have been offered CyBorD induction (consisting of weekly cyclophosphamide + bortezomib 1.3-1.5 mg/m² + dexamethasone), ASCT and thalidomide maintenance if available. Maintenance with thalidomide was replaced by lenalidomide whenever feasible in 2012. Using the database, we have analysed the results of myeloma therapy in the real-world setting in the following groups of patients receiving first-line therapy:

- 1) Overall survival in ASCT patients before and after the availability of bortezomib-based induction therapy before ASCT.
- 2) Response rates after 4 cycles of CyBorD and at day 100 post-ASCT in transplant-eligible patients.
- 3) Progression-free survival in ASCT patients treated with CyBorD induction and lenalidomide maintenance given after transplant until disease progression.
- 4) Outcomes of patients with high-risk myeloma receiving tandem ASCT.

The Princess Margaret Database platform has now been adopted by and expanded into the Myeloma Canada Research Network (MCRN) Database, and legacy data will be forthcoming in 6000 patients from 3 centres; an additional 10 centres are beginning to collect prospective data, including details and toxicity of sequential regimens. Soon, both patient-reported outcomes and novel biologic correlates performed in several laboratories across Canada will be integrated with the clinical information in the database. The database will be used to define gaps in care and generate hypotheses for testing in prospective clinical trials conducted by the MCRN.

089. Management of relapsed/refractory multiple myeloma

Reece D

Princess Margaret Cancer Centre / University of Toronto

Although considerable progress has been made in the management of newly diagnosed myeloma, virtually all patients eventually relapse. Until a cure has been discovered, the current management involves the judicious use of sequential regimens, each given for maximal benefit in controlling the disease for as long as possible before another relapse occurs. Recently, multiple phase 3 studies have compared the older “doublet” regimens—either lenalidomide + dexamethasone or lenalidomide + dexamethasone—with newer combinations, most often triplet regimens in which a new proteasome inhibitor or monoclonal antibody is added to the doublet backbone. As these regimens become available to haematologists, there is considerable uncertainty regarding the optimal selection of therapy for a given patient. Unanswered questions include the ideal sequencing of regimens and whether first-line therapy should be adjusted to allow potential access to potent combinations now available only in the relapsed/refractory setting.

This session will provide an overview of newer therapeutic options for recurrent myeloma, emphasizing the results of phase 3 trials. Factors involved in the selection of therapy for relapse, including the disease heterogeneity, patient-related factors and toxicity considerations, will be reviewed. Alternative strategies under investigation by the Myeloma Canada Research Network, such as development of less expensive triplet regimens and evaluating the addition of a new agent only “on demand” will be presented. Finally, issues relevant to first relapse compared with advanced relapse will be discussed.

090. Efficacy of daratumumab-bortezomib-dexamethasone (DVd) vs bortezomib-dexamethasone (Vd) based on treatment-free interval and prior treatments: CASTOR

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Aim: To assess efficacy of DVd versus Vd in subgroups based on prior treatment history in CASTOR.

Methods: Relapsed or refractory myeloma patients who received ≥ 1 prior line of therapy (LOT) received 8 cycles of Vd (Days 1, 4, 8, 11 for bortezomib 1.3 mg/m² SC; Days 1-2, 4-5, 8-9, and 11-12 for dexamethasone 20 mg PO) \pm daratumumab (16 mg/kg IV QW in Cycles 1-3, Q3W for Cycles 4-8, then Q4W until progression). High cytogenetic risk (determined using next generation sequencing) was defined as having t(4;14), t(14;16), or del17p abnormalities.

Results: After 13.0 months median follow-up, DVd prolonged PFS for patients treated >12 months (median NR vs 9.4 months; HR, 0.27; 95% CI, 0.17-0.43; $P<0.0001$) or ≤ 12 months (median 10.3 vs 5.2 months; HR, 0.34; 95% CI, 0.24-0.48; $P<0.0001$) after last LOT. ORR for DVd vs Vd was 91% vs 83% ($P=0.0632$) in the >12 month subgroup and 77% vs 49% ($P<0.0001$) in the ≤ 12 month subgroup.

Among bortezomib-pretreated patients, PFS (median: 12.4 vs 6.7 months; HR, 0.37; 95% CI, 0.28-0.50; $P<0.0001$), ORR (81% vs 60%; $P<0.0001$) and MRD-negative rates (6% vs 1% at 10^{-5} ; $P=0.0056$) were improved with DVd vs Vd.

In patients refractory to lenalidomide at last prior LOT, PFS (median: 9.3 vs 4.4 months; HR, 0.36; 95% CI, 0.22-0.58; $P<0.0001$), ORR (81% vs 50%; $P=0.0021$), and MRD-negative rates (9% vs 0% at 10^{-5} ; $P=0.0082$) were significantly improved with DVd vs Vd.

Additional efficacy data, including analyses by cytogenetic risk and MRD status, will be presented.

Conclusions: DVd is superior to Vd regardless of time since last therapy, prior exposure to bortezomib, or refractoriness to lenalidomide.

091. Progression from newly diagnosed to relapsed/refractory myeloma causes significant alterations in the CD4⁺ Treg population

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¹Australian Cancer Research Foundation Laboratory, Royal Melbourne Hospital / VCCC, Melbourne, Australia, ²Department of Medicine, University of Melbourne, Melbourne, Australia, ³Peter MacCallum Cancer Centre, Melbourne, Australia

Introduction: Immune compromise is a recognized complication of multiple myeloma (MM), aging and anti-MM therapies used throughout the course of the disease. It has been reported that patients with relapsed/refractory MM (RRMM) have marked CD4⁺ T lymphopenia that is not present in newly diagnosed MM (NDMM) and does not recover with successive cycles of lenalidomide and dexamethasone (len/dex) treatment^{1,2}. However, the proportion of Tregs within the CD4⁺ T cell population increased with successive cycles of treatment and approached normal range by cycle 9¹. We hypothesized that this represented homeostatic proliferation of peripherally-derived Tregs (pTreg) as opposed to thymic production of naturally-derived Tregs (nTreg), which can be differentiated by the methylation status of the T-cell specific demethylation region (TSDR) in the Foxp3 gene³.

Methods: We analysed 5 paired samples from patients with NDMM and RRMM (before and after len/dex) and 5 age-matched normal donors. Tregs were FACS sorted and DNA was extracted and bisulfite converted. The TSDR was PCR amplified and transformed into chemically competent *E.coli*. Individual colonies were picked and their DNA plasmids were sequenced. Cytosine matches/mismatches at CpG sites in the TSDR were identified and % methylation calculated.

Results: The TSDR methylation status of Tregs from baseline NDMM samples was largely unmethylated and similar to age-matched controls, i.e. predominantly nTregs, whereas baseline RRMM samples were largely methylated, i.e. predominantly pTregs. Len/dex treatment in both NDMM and RRMM patient did not alter the respective phenotypes.

Conclusions: This study adds evidence that the character of the CD4⁺ T cell population is radically altered in RRMM, compared with NDMM and age-matched controls. This bears relevance, as pTregs are likely to be derived from senescent cells that are not only functionally different to recent thymic emigrants⁴ but may also exhibit plasticity⁵, leading to compromise of the effector T cell population in RRMM.

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092. EuroFlow MRD detection and outcomes for patients undergoing transplantation for high risk myeloma.

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Aim

Minimal residual disease measured by flow cytometry (MRD) has been shown to correlate with quality of response post treatment for myeloma. We audited our use of the EuroFlow myeloma panel following autologous (autoHSCT) and allogeneic transplantation (alloHSCT) for high risk myeloma.

Method

Retrospective audit of all myeloma patients who underwent transplantation at our unit between October 2014 and February 2017.

Results

90 patients underwent autoHSCT. 24% of the patients (13 of 55 assessed) were MRD negative subsequently.

31 patients underwent non-myeloablative alloHSCT, with the intention to have regular EuroFlow MRD monitoring from 3 months onwards. The median follow-up was 18.9 months. MRD was negative in 29% of patients at 3 months and 63% at 12 months.

MRD positivity post alloHSCT at 6 months was predictive of outcome. The rate of progression at 1 and 2 years respectively was 14% (1 out of 7) and 25% (1 of 4) for patients MRD negative at 6 months vs 50% (5 of 10, $p=0.30$) and 100% (7 of 7, $p=0.02$) for those MRD positive.

At 3 months the rate of progressive/relapsed disease was similar between those who were MRD negative and positive. 6 patients (30%) who were MRD positive subsequently became MRD negative (without specific anti-myeloma treatment).

The overall rate of progressive/relapsed disease post alloHSCT at 1 year was 38% (8 of 21 patients) and at 2 years 73% (11 of 15 patients). This rate was lower for patients who were MRD negative pre alloHSCT (14%, 1 patient out of 7, $P=0.15$).

Conclusion

MRD negativity pre alloHSCT, though not at 3 months, was associated with a reduced rate of relapse/progression. A number of patients changed from MRD positive to negative suggesting a graft vs myeloma effect. EuroFlow MRD positivity at 6 months was associated with a high rate of relapse/progression.

093. Renal amyloidosis: it's not all AL

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Aim

To identify cases of renal amyloidosis incorrectly subtyped as light chain amyloidosis (AL) and highlight patient and disease characteristics to avoid incorrect management.

Background

Amyloidosis involves the deposition of misfolded protein fibrils in multiple organs. AL is the most common in chronic kidney disease (CKD) but subtype diagnosis is essential for prognosis and treatment.

Methods

We reviewed the Victorian and Tasmanian Amyloidosis Service (VTAS) database for cases and clinical characteristics of renal AL and non-AL.

Results

The VTAS database identified 137 patients, 38 with renal amyloidosis: 31 AL-type (82%), 2 Leukocyte Chemotactic Factor 2 (LECT2) (5%), 1 Fibrinogen a- α chain (3%), and 4 AA (10%).

2 patients with LECT2 were Egyptian, had isolated renal involvement with non-nephrotic-range proteinuria and were diagnosed using mass spectrometry and LECT2 immunohistochemistry. One progressed to end stage kidney disease (ESKD); the other has stable Stage IIIB CKD.

Fibrinogen a- α chain is a nephrotic, hereditary condition where amyloid deposits are characteristically restricted within expanded glomeruli, as with our patient. Diagnosis was established using mass spectrometry and genetic testing.

AA, secondary to infection/inflammatory states, was identified in 4 patients with nephrotic-range proteinuria diagnosed on immunohistochemistry. The cases of AA included Familial Mediterranean Fever (FMF) treated with colchicine (stable renal function); a rare and lethal autoimmune inflammatory skin condition; untreated metastatic Carotid Body Paraganglioma progressed to ESKD; and unknown aetiology with progressive CKD.

Conclusion

Almost 20% of patients with renal amyloidosis don't have AL. Ethnicity, isolated renal involvement and histological findings should prompt investigation of non-AL, as chemotherapy, with associated toxicities, has no therapeutic role. Patients may benefit from an Australian Amyloidosis Network centre to confirm the subtype and access novel treatments.

094. Allogeneic Stem Cell Transplantation in Multiple Myeloma

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Aim

The role of allogeneic transplantation in multiple myeloma remains controversial. We assessed the outcomes of allogeneic stem cell transplantation offered in the course of treatment for multiple myeloma in our centre.

Method

From 1/03 – 6/17, 221 myeloma patients (pts) received autologous only stem cell transplantation (auto-SCT), and 21 patients received allogeneic (allo only or auto-allo) transplantation (allo-SCT) at our centre. Outcomes for the two groups were assessed from diagnosis as well as a landmark analysis from first relapse.

Results

For the entire cohort, median age at diagnosis was 59 (22 - 69) yrs. 60.3% (146/242) were male. Median follow up was 3.8 (0.44 – 18.05) yrs. KM estimates of 7 yr disease free (DFS) and overall (OS) survival were 44.2% and 71.5% respectively. Allo-SCT were younger, median age 56 (40-66) vs 60 (22-69) yrs ($p=0.006$) and had higher TRM (28.5% vs 3.7%, $p<0.005$) than auto-SCT. From first diagnosis, allo-SCT had inferior 7 yr DFS ($p=0.004$) with a trend towards lower OS ($p=0.09$) vs auto-SCT. Landmark OS analysis at first relapse showed no advantage for allo-SCT vs second auto-SCT ($p=0.75$), though small numbers limited the utility of this analysis.

Conclusion

In our single centre study, there was no discernible advantage to proceeding with allo-SCT in the management of myeloma. Allo-SCT pts were younger, experienced higher TRM and inferior DFS, but landmark analysis at first relapse could show no OS advantage for allo-SCT in this setting. Future collaborative trials will be required to assess which subset of myeloma pts, if any, may benefit from allo-SCT.

095. Determinants of survival following allogeneic stem cell transplantation for multiple myeloma: a single centre experience.

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Aim

Allogeneic stem cell transplantation remains the only curative therapy for myeloma although data regarding its safety and efficacy is conflicting¹. We investigated the determinants of overall survival and relapse free survival following allogeneic stem cell transplant for multiple myeloma at our centre.

Method

Retrospective analysis of all patients with MM undergoing first allogeneic stem cell transplantation from 2000 to 2017. Conditioning was with non-myeloablative regimes or reduced intensity conditioning regimens. Data was interrogated using multivariate analysis with the STATA data package. Patient and disease characteristics analysed included demographics, donor type, conditioning type, T cell depletion, lines of treatment and disease status prior to transplant.

Results

51 patients were identified. Median overall survival from the time of transplant is 3.8 years. Patients with either stable, persistent MM or progressive MM at the time of transplant had a hazard ratio for death of 2.9 ($p < 0.005$). This strong association holds despite controlling for potential confounders in multivariate analysis, such as conditioning regimen and year of transplant. Conversely, the number of lines of treatment prior to transplant does not affect overall survival. In this analysis we could not demonstrate any clear associations with relapse free survival, but show that use of thymoglobulin has an association with relapse that approaches significance (HR 2.1 $p = 0.07$).

Conclusion

Our data supports the importance of disease status as a determinant of overall survival post-allogeneic stem transplant and may implicate T cell depletion in abrogating a graft vs myeloma effect.

References

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096. Differences in genetic profile underscore aggressiveness of therapy-related myeloid neoplasms (T-MN) compared to Primary-MDS (P-MDS)

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Aim: T-MN occurs after chemo (CT) and/or radiotherapy (RT) exposure and has poor prognosis. Few studies based on small T-MN cohorts showed only 50-60% T-MN harbour mutations compared to 80-90% P-MDS. This study compares genetic profiling of P-MDS and T-MN.

Method: Clinical/laboratory data of 744 P-MDS and 147 T-MN and 43-gene panel sequencing data from a subset (135 P-MDS/101 T-MN) from South-Australian MDS registry were analysed. Chi-square and Fisher's exact tests were used for comparison.

Result: In T-MN, lymphoproliferative (38%) and prostate cancers (15%) were most frequent primary neoplasms. Sixty-three (43%) received CT, 34 (23%) RT, and 48 (33%) CT±RT. Poor risk cytogenetics (per International Prognostic Scoring System) were significantly higher in T-MN post-CT compared to RT and P-MDS (Fig.1A, $p < 0.0001$).

Mutations in 86% P-MDS and 94% T-MN cases were detected (Fig.1B). Mutations in >1 gene were detected in 70% of P-MDS and 76% T-MN. Despite similar mutational frequency and burden between P-MDS and T-MN, the pattern was different. Spliceosome mutations were significantly more common in PMDS (53% vs 26%, $p < 0.0001$) while *TP53* (32% vs 7%, $p < 0.0001$) and transcriptional factors (35% vs 20%, $p < 0.05$) were enriched in T-MN. Significantly more *TP53*^{mut} T-MN had complex karyotype (84%) compared to *TP53*^{wt} (7%, $p < 0.001$, Fig.1C). Nineteen percent P-MDS and 11% T-MN showed >1 mutation in *TET2* (mostly with similar variant allele frequency) suggesting genomic mutagenicity induced by the initial *TET2* mutation. Similarly, multiple mutations in the same gene were also detected in *KRAS*, *RUNX1* and *TP53* (Fig.1D).

Conclusion: Contrary to published literature, we found high mutation frequency in a large T-MN cohort. High risk cytogenetics and *TP53*/transcriptional factor mutations were significantly more frequent in T-MN compared to P-MDS while spliceosome mutations were more frequent in P-MDS. These genetic differences underscore the aggressiveness of T-MN and different therapeutic strategies are required for T-MN and P-MDS.

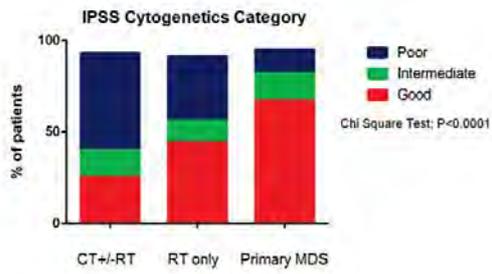


Fig.1A T-MN with prior CT have significantly higher frequency of IPSS poor risk cytogenetics

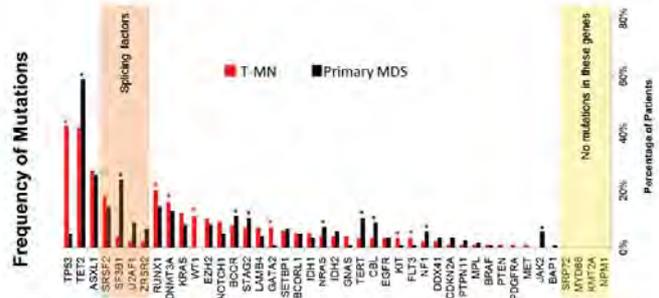


Fig.1B The differential spectrum of mutations in T-MN & primary MDS

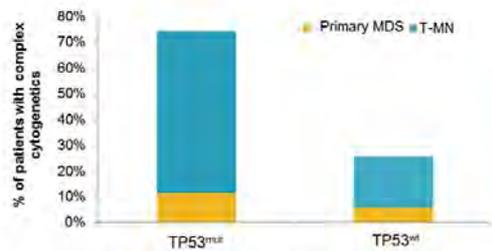


Fig 1C TP53 mutations are enriched in patients with complex cytogenetics

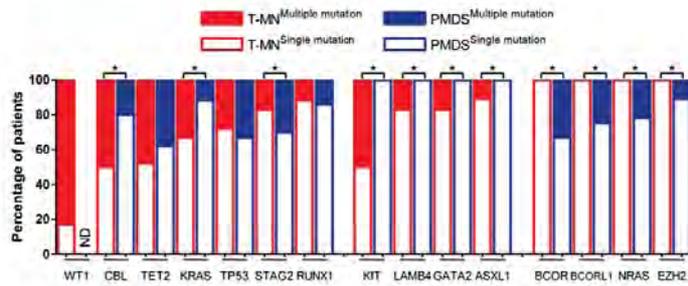


Fig. 1D Multiple mutations in the same genes were detected in primary MDS and T-MN (Fisher's exact test)

097. Low Risk Revised IPSS does not predict overall survival: retrospective review of 262 patients.

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Aim

To investigate *real-world* survival outcomes of patients with Myelodysplastic Syndrome (MDS) who are diagnosed and treated in an Australian context.

Method

Ethics and governance was granted as part of quality improvement at 3 tertiary hospitals. Cases were identified through interrogation of the WA health department database by hospitalisations records between 2008 and 2015. Individual case records were reviewed for bone marrow pathology, diagnostic full blood picture (FBP), cytogenetics, Charlson co-morbidity index, transfusion history and demographics. WHO 2008 diagnostic criteria were applied to confirm the diagnosis of MDS and cases were retrospectively assigned a Revised IPSS score from the time of diagnosis (not *admission*). Statistics are descriptive in nature prepared using SPSS version 24.

Result

341 patients were identified, of which 79 cases were excluded based on bone marrow pathology being non-confirmatory of MDS. The median age of the cohort was 74 years with a male to female 2.27. The cohort characteristics are shown in Table 1. 13% could not be categorised by R-IPSS due to lack of available data (e.g. technical failure of cytogenetics). The case-mix was similar across all sites, with a trend towards more high risk R-IPSS cases at one hospital (possible referral bias), and a trend towards increased survival at the allogeneic transplant site (p 0.074).

Conclusion

In this retrospective review, we have identified those patients considered “very low” and “low” risk by R-IPSS for MDS progression (and death), to have lower than expected overall survival. This cohort have median OS of <3.3 years, compared with large international registry data of 5.3 – 8.8 years (1). Intermediate to high risk cases appear to have OS outcomes similar to predicted by R-IPSS. Further evaluation of Australian demographic and contextual factors should be considered in prognostication of patients with lower risk MDS.

"No conflict of interest to disclose".

Table 1: Cohort Characteristics by IPSS-R group

R-IPSS group		n	% cohort	Median Age	Range	n, deceased	Median OS, months	Range	n, missing f/u data
Very Low Risk R-IPSS		32	12%	75	64-88	15	39	0-114	2
Low Risk R-IPSS		68	26%	77	47-92	40	27	1-129	3
Int Risk R-IPSS		47	18%	75	45-90	35	19	0-86	1
High Risk R-IPSS		41	16%	69	23-96	35	15	0-82	1
Very High Risk R-IPSS		39	15%	68	33-90	31	12	1-155	3
Unclassified		35	13%	72	32-88	26	30	0-185	2

1. Greenberg P, Tuechler H, SChanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised International Prognostic Scoring System for Myelodysplastic Syndromes. *Blood*. 2012;120(12):2454 - 65.

098. Abnormal G8 predicts increased mortality and health-resource utilisation in elderly Azacitidine-treated myelodysplastic syndrome (MDS) patients

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Aim: Though elderly individuals of same age have variable physical, cognitive functions and comorbidities, treatment decisions are still based on disease biology and chronological age. This pilot project assesses the contribution of a comprehensive geriatric assessment (CGA) in the management of elderly MDS patients.

Method: After informed consent, patients underwent a CGA by nurse case manager working collaboratively with the geriatrician and haematologist. CGA was performed using well-defined tools - ECOG, ADL, iADL, GDS, polypharmacy, nutritional assessment, MMSE, falls, medication adherence and comorbidity. As CGA takes 80-90 minutes, two geriatric screening tools (VES-13 and G8) were also used.

Results: Over three years, 76 patients ≥ 65 years were enrolled. Twenty-seven (35%) and 49 (64%) patients were treated with Azacitidine (Aza) and supportive care respectively. Patients were classified as G8^{Abnormal} (score ≤ 14 ; n=51) or G8^{Normal} (score >14 ; n=25). The sensitivity and specificity of G8 in detecting a dependency in at least one CGA domain was 80% and 63% respectively, while that of VES-13 was 50% and 85% respectively. Furthermore, G8 also predicted health resource utilisation while VES-13 did not. Compared to G8^{Normal} patients, G8^{Abnormal} patients required significantly more frequent and longer hospital admissions ($p < 0.001$). Importantly, median overall survival (OS) of Aza-treated patients was significantly poor in G8^{Abnormal} (n=21) patients compared to G8^{Normal} patients (n=7, $p < 0.003$; Fig 1A). All G8^{Normal} tolerated at least 5 cycles of Aza compared to only 22% of G8^{Abnormal} patients. Interestingly, median OS between G8^{Abnormal} and G8^{Normal} group managed with supportive care was not significantly different (Fig 1B).

Conclusion: This study demonstrates that geriatric screening G8 is not only sensitive in detecting abnormality in CGA but also predicted poor survival, lower treatment completion rates and higher health resource utilisation in Aza-treated patients. Hence, geriatric assessment should be included in the treatment decision making process for MDS patients.

099. Azacitidine treated myelodysplastic syndromes (MDS) patients are at higher risk of bacteraemia

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Aim

Despite being a major cause of morbidity and mortality in MDS, there is little recent data on epidemiology of infections. This study evaluates the frequency and causative organisms of bacteraemia in patients registered in the South Australian MDS (SA-MDS) registry.

Method

A retrospective analysis of febrile illness and bacteraemia in 996 MDS patients. Cox regression analyses were used to determine predictors of bacteraemia.

Results

737/996 (74%) of MDS patients had at least one febrile illness and 199 (20%) patients had 319 bacteraemic episodes. The most common organisms were gram-negative bacilli (GNB; n=138, 43%), viridans streptococci (n=41, 12%), *Staphylococcus aureus* (n=34, 11%) and enterococcus (n=30, 9%). The proportion of GNB bacteraemias increased (49% vs. 38% vs. 29% after 2012, 2006-2011 and 1999-2005 respectively) without increased resistance to commonly used antibiotics. The most common GNB were *E. coli*, *Klebsiella* and *Pseudomonas* species. There were 31 episodes of enterococcus bacteraemia with significant increase in the proportion of vancomycin resistant enterococcus (VRE) bacteraemia after 2011 (p<0.001).

Bacteraemia was significantly increased in patients treated with allogeneic stem cell transplantation (n=29) or chemotherapy (n=112) compared to azacitidine (AZA; n=206) or supportive care (SC; n=625, p<0.001). Notably, AZA treated patients have higher incidence of bacteraemia than SC patients; matched for IPSS-R (17% vs 9%; p=0.008). Majority of bacteraemia in AZA treated patients was during first 6-cycles (22/37; 60%).

Revised International Prognostic Scoring System (IPSS-R) risk groups, red blood cell-transfusion dependency status and monocytes at diagnosis were independent predictors of bacteraemia (p<0.001). Cumulative incidence of bacteraemia was significantly higher in IPSS-R Very High compared to Very Low risk group (21% vs. 5%; p=0.005).

Conclusion

Febrile illness and bacteraemia are common in MDS and increasingly caused by GNB and VRE. Compared to SC, AZA treated patients are at higher risk of bacteraemia mainly during initial 6-cycles.

100. ASXL1 c.1934dup;p.Gly646Trpfs*12 – a true somatic alteration requiring a new approach

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Aim

Truncating mutations of *ASXL1* are associated with an inferior overall survival in a variety of myeloid malignancies including AML, MDS, CMML and MF.

ASXL1 NM_015338.5:c.1934dup;p.Gly646Trpfs*12 (*ASXL1* c.1934dupG), the most common truncating *ASXL1* mutation (approximately 50% of cases) occurs via the duplication of a single guanine within an eight base pair mononucleotide guanine repeat. Some authors have claimed that *ASXL1* c.1934dupG is a sequencing artefact due to replication slippage.

This study aimed to prove that *ASXL1* c.1934dupG is a true somatic alteration and to determine the optimal testing strategy for its detection.

Method

A novel qRT-PCR assay using a mutation-specific primer was developed for the detection and quantification of *ASXL1* c.1934dupG.

A cohort of 186 samples from patients with myeloid malignancies was tested via amplicon-based massively parallel sequencing (MPS), Sanger sequencing (SS) and fragment analysis (FA). A subset of this cohort together with additional patient samples was tested by qRT-PCR.

Result

ASXL1 c.1934dupG was proven to be a true somatic alteration by demonstrating differential annealing of the mutation-specific primer within mutated versus wild type DNA.

ASXL1 c.1934dupG accounted for 44.23% (23/52) of truncating *ASXL1* mutations in patient samples. MPS was inferior to the gold standard (SS/FA) for the detection of *ASXL1* c.1934dupG (sensitivity 86.96%, specificity 93.87%).

qRT-PCR had an improved limit of detection compared with SS/FA (3% vs 12.5% mutation burden). The superior sensitivity of qRT-PCR has been utilised in patient samples to detect otherwise undetectable *ASXL1* c.1934dupG containing subclones at diagnosis as well as to monitor measurable residual disease after allogeneic stem cell transplantation.

Conclusion

ASXL1 c.1934dupG is a true somatic alteration. Due to the suboptimal sensitivity of amplicon-based MPS for *ASXL1* c.1934dupG detection, we recommend the synchronous use of an adjunctive method for this purpose. The novel qRT-PCR assay described herein represents a novel method of *ASXL1* c.1934dupG detection, the greater sensitivity of which may add value in certain clinical contexts.

101. Predicting infections in myelodysplastic syndromes: A 12-year population-based study of epidemiology, risk factors and outcomes.

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Background/Aim: Despite infection being a common cause of morbidity and mortality in MDS, studies describing infections are predominantly focused on single centres or selective subgroups (high-risk MDS; patients receiving hypomethylating agents), with limited data on the impact of clinical factors (eg.comorbidities, transfusion dependence). We aimed to describe the epidemiology of infections in all MDS patients admitted to hospital in Victoria.

Methods: All hospital inpatient episodes with a diagnosis of MDS between 1998-2010 in Victoria including demographic and clinical data were identified via the Victorian Admitted Episodes Dataset (VAED), generated from discharge coding from all public and private hospitals. MDS diagnoses were confirmed by linkage with the Victorian Cancer Registry. Infections were identified through International Classification of Diseases (ICD) codes in VAED.

Results: Of 3797 MDS patients identified, 52.3% (n=1987) had one or more admissions with infection. Patients with infection were more likely be red cell transfusion dependent (65.4% vs. 25.7%,p<0.001), cytopenic at MDS diagnosis (anaemia in 32.7% vs.19.1%,p<0.001; thrombocytopenia in 3.6% vs.2.2%,p=0.010; neutropenia in 12.4% vs.4.8%,p< 0.001), have AML transformation (15.2% vs.3.5%, p< 0.001) and comorbidities (Table 1).

Table 1: Comorbidities in patients with and without infection

Comorbidity	Patients with infection (N=1987), n(%)	Patients without infection (N=1810), n(%)	p-value
Renal disease	331(16.8%)	156(8.6%)	<0.001
Peripheral vascular disease	113(5.7%)	58(3.2%)	<0.001
Pulmonary disease	317(16%)	123(6.8%)	<0.001
Myocardial infarction	193(9.7%)	93(5.1%)	<0.001
Chronic liver disease	28(1.4%)	12(0.7%)	0.024
Diabetes mellitus	322(16.2%)	143(7.9%)	<0.001
Stroke	241(12.1%)	104(5.7%)	<0.001
Cardiac failure	450(22.6%)	179(9.9%)	<0.001

Bacterial infections occurred in 1959 patients (51.6%); fungal infections in 297 (7.8%). Documented pathogens included candida (n=213, 5.6%), Gram-negative sepsis (n=166, 4.4%), streptococcus (n=56, 1.5%), aspergillus (n=34, 0.9%), staphylococcus (n=28, 2%), mycoses (n=22, 0.6%) and anaerobes (n=8, 0.2%). Sites of infection included lung (n=1240, 32.7%), gastrointestinal tract (n=904, 23.8%), renal tract (n=876, 23.1%), septicaemia (n=499, 13.1%) and central nervous system (n=21, 0.6%). Patients with infection had lower overall survival (18.2% vs 35.7%, p<0.01).

Conclusion: Over half of MDS patients admitted to hospital experienced infection. Bacteria and candida are common pathogens. This is the first study to demonstrate the impact of individual comorbidities on infection risk at population level. Future directions include trials to test prophylactic and therapeutic approaches to infection.

105. Targeted sequencing of myeloid malignancies: a pilot study

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Aim

The importance of well characterised haematological malignancies by molecular means is well known. Former methods can be somewhat laborious, varied and not necessarily sensitive. Next generation sequencing has brought to research and now to the clinic a more standardised method to detect variants.

This validation study presents a targeted panel as a precursor to introduction for clinical use.

Method

A cohort of 35 individuals presenting with haematological diseases such as acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPNs) was chosen for it's range of previously characterised variants tested in our laboratory.

DNA extracted from bone marrow was amplified and libraries prepared using the Custom AmpliSeq Myeloid Early Access panel, containing 30 genes. Sequencing was performed using Hi-Q View on the Ion Torrent PGM (Thermo Fisher Scientific).

Ion Reporter software, incorporating Variant Caller and Coverage Analysis was used to give read depth and COSMIC variant ID. Additionally, IGV (Broad Institute) was employed.

Result

Targeted sequencing indicated not only a high concordance with results obtained by alternate methods but new variants were detected, e.g. GATA2, RUNX1, TET2, TP53. To date, Sanger sequencing has confirmed many of these variants.

Three driver mutations involved in MPNs, JAK2V617F, CALR and MPL, consisting of single base variants, insertions and deletions, were mutually detected by all methods.

The most common molecular variants in AML and MDS are found in the FLT3, NPM1, CEPBA and IDH 1/2 genes, the latter three giving highly concordant results with alternative methods. Sequencing of the ITD within the FLT3 gene is problematic when a double ITD or a duplication greater than ~60bp is present.

Conclusion

This Custom Myeloid panel has a gene number and primer specificity capable of accurately detecting variants in genes not only well known for their prognostic influence but also those genes seen in prediagnostic clonal involvement of myeloid malignancies

106. miR-10a inhibition sensitizes nucleophosmin 1-mutant acute myeloid leukaemia cells to cytarabine

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Aim

Mutations in the nucleophosmin 1 (NPM1^{c+}) gene represent one of the most common genetic aberrations in acute myeloid leukaemia (AML), occurring at a frequency of approximately 30%. We and others have shown that NPM1^{c+} AML exhibits a distinct microRNA (miR/miRNA) expression profile, notably characterised by the striking upregulation of miR-10a. We previously demonstrated that miR-10a functions as a pro-survival molecule, and directly targets several cell cycle modulators known to have roles in chemotherapy responsiveness including p21 regulator TFAP2C, RB1 regulator RB1CC1, and cell cycle regulator GTF2H1. We aimed in the current study to determine whether inhibition of miR-10a could sensitize NPM1^{c+} AML cells to chemotherapy via modulation of cell cycle.

Method

The miR-10a-overexpressing NPM1^{c+} cell line OCI-AML3 was stably transduced with either a miR-10a inhibitor or scrambled control. Sensitivity to cytarabine was determined *in vitro*, as were differences in cell cycle, proliferation, and apoptosis. To confirm findings *in vivo*, NSG mice were engrafted with each transduced cell line and treated with either cytarabine or saline (IP) for 3 weeks. Differences between treatment groups were quantified by T-test and ANOVA, and survival analyses performed using Cox Regression and Kaplan-Meier plot.

Result

Inhibition of miR-10a combined with cytarabine in OCI-AML3 cells *in vitro* led to a significant perturbation of the cell cycle, with the majority of cells arresting soon after treatment (Day0-4) unlike controls. This led to significantly decreased proliferation and increased apoptosis at later timepoints (Day8-12; both $p < 0.05$). These effects were reflected *in vivo*, with significantly improved overall survival in mice engrafted with miR-10a-inhibited OCI-AML3 cells ($p < 0.01$) and fewer leukaemic cells in the bone marrow ($p < 0.05$) compared to controls following cytarabine treatment.

Conclusion

This study demonstrated that inhibition of the pro-survival microRNA, miR-10a, sensitizes NPM1^{c+} OCI-AML3 cells to cytarabine, likely via modulation of key cell cycle regulators.

107. How far have we come in the world of Cutaneous Lymphoma in Australia

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Aim

Mycosis Fungoides (MF) and other cutaneous lymphomas can be difficult to diagnose, manage and treat. The Multidisciplinary Cutaneous Lymphoma Clinic was first established at St Vincent's Hospital, Melbourne. Initially this clinic was predominantly developed to assist in the diagnosis and treatment of patients with MF. This service has expanded to include all patients with cutaneous lymphomas, and now incorporates a weekly multidisciplinary service based at Peter MacCallum Cancer Centre in Melbourne.

Method

This descriptive paper is an overview of 1174 patients who have been through the Cutaneous Lymphoma Service. On average 53 patients are referred every year.

Results

The current National Cutaneous Lymphoma Database has expanded to include cutaneous B Cell types as well as cutaneous T cell lymphoma. From 1977-1995, 107 patients were recorded with various stages of MF. In 2017, there are 1158 recorded patients with cutaneous lymphoma in the database, including 685 patients with MF.

In 1997 time of skin eruption to diagnosis was 7.71 years to 9.23 years. In 2014, time of skin eruption to diagnosis was 3 years in conditions such as advanced stage MF. Advances in molecular diagnostic tools and histopathology have reduced the time to diagnosis.

In 1997 response to treatments was used as a marker of disease response. In 2017, we have incorporated the criterion of time to next treatment as a tool to better monitor and prognosticate disease.

Conclusion

The Cutaneous Lymphoma Service continues to grow and evolve. The Melbourne based service runs multidisciplinary clinics, online clinical service meetings, and conducts research relevant to the needs of patients living with cutaneous lymphomas. The complexity of cutaneous lymphomas and the need for more collaborative research is unchanged; however, this is being addressed through such initiatives as PROCLIP and the ACLN (Australian Cutaneous Lymphoma Network).

No conflict of interest to disclose.

108. A droplet digital PCR method for sensitive quantification of the JAK2 V617F mutation

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Introduction

JAK2 V617F is the most common mutation detected in Ph-negative myeloproliferative neoplasms (MPN). Droplet digital PCR (ddPCR) is a quantitative and highly sensitive method of detecting point mutations which can be applied to various clinical scenarios.

Aim

To report our experience with using ddPCR for JAK2 V617F and its clinical utility.

- Validation of the methodology by comparing with orthogonal methodologies
 1. Detection low level mutations in patients negative by conventional PCR
 2. Monitoring response to therapy

Method

DNA is extracted from whole blood or bone marrow of patients with suspected or confirmed MPN. Clinical information is obtained from the requesting physician and patient records. PCR is then performed on DNA following droplet generation. Using fluorescent probes for the wild type and mutant alleles enables quantification using flow cytometry.

Result

ddPCR was validated against several benchmarks and was shown to be a robust and reproducible method. The limit of detection approached 0.01%. One patient with the diagnostic conundrum of persistent mild thrombocytosis and equivocal marrow findings, had a diagnosis of JAK2-V617F positive essential thrombocythemia established by detecting a clinically relevant transcript level of <1%. Others have had monitoring of transcript changes following therapy (interferon, allogeneic SCT) which has informed clinical decisions.

Conclusion

Highly sensitive ddPCR can detect clinically relevant levels of JAK2-V617F mutant clones missed by conventional PCR. ddPCR should be considered in all patients with triple-negative MPN. Quantitative results can be used for monitoring post-allograft and can inform clinical decision making for patients on active therapy.

No conflict of interest to disclose

109. Quantification of autophagy in Bortezomib-resistant multiple myeloma by field emission scanning electron microscopy (FESEM)

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Hypothesis

Autophagy is a mechanism of resistance to Proteasome Inhibitors (PI) in Multiple Myeloma (MM).

Aim

To use FESEM to assess and quantify autophagy as a mechanism of resistance to Proteasome Inhibitors in MM.

Background

Proteasome Inhibitors, such as bortezomib, are effective in a significant proportion of MM. However, acquired resistance to proteasome inhibitors is common. Understanding the mechanism of resistance may lead to targeted therapies that could overcome resistance.

Proteasome inhibitors are known to induce the unfolded protein response (UPR) and autophagy, via cell stress. We have previously shown by scanning electron microscopy that the UPR is down regulated in acquired resistance to Bortezomib. Therefore, it is possible that resistance is acquired through induction of autophagy, with cells adapting to become less reliant on the UPR.

By using FESEM and image analysis we quantify the activity of autophagy in Bortezomib sensitive and resistant MM cell lines. The resistant MM cell line has been derived by long-term adaption to Bortezomib.

Method

FESEM images of cross sections of MM sensitive and resistant cell lines were captured. 30 sensitive and 30 resistant MM cells of similar mitotic phase were selected. Autophagosomes were categorised based on size; small (10 microns), medium (14 microns) and large (24 microns). These structures are counted and multiplied by the area. The total areas reflect the overall autophagic activity.

Result

Autophagy is significantly increased in MM resistant to proteasome inhibitors.

Conclusion

This study suggests that autophagy is a mechanism of resistance to proteasome inhibitors. FESEM and computerised image analysis are useful tools to quantify autophagy in cancer. Analysis of primary MM samples is underway to validate the *in vitro* finding.

110. Transplant-ineligible (TNE) patients with newly diagnosed multiple myeloma (NDMM): FIRST trial final survival analysis

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Aim

The FIRST trial demonstrated lenalidomide plus low-dose dexamethasone until progression (Rd continuous) vs melphalan, prednisone, and thalidomide (MPT) or Rd for 18 cycles (Rd18) improved outcomes for TNE patients with NDMM. We present the prespecified final overall survival (OS) analysis.

Methods

Patients were randomized to Rd continuous (n=535), MPT (n=547), or Rd18 (n=541). Progression-free survival (PFS) was the primary endpoint. Secondary endpoints included OS, overall response rate, time to second therapy (TTST), and safety. Primary comparison was Rd continuous vs MPT.

Results

As of Jan 2016 (final OS analysis), 52 patients (Rd continuous arm) continued treatment. Median follow-up was 67.0 months. Median OS was significantly longer with Rd continuous vs MPT (59.1 vs 49.1 months; P=.0023); 62.3 months for Rd18. Nearly 3 years after the original analysis, a PFS advantage remained with Rd continuous vs MPT (median PFS 26.0 vs 21.9 months, P<.00001); 21.0 months for Rd18. Four-year PFS was 32.6%, 13.6%, and 14.3%, respectively. Second-line treatment was initiated in 299, 381, and 377 patients, respectively. Median TTST was 36.7, 26.7, and 28.5 months with Rd continuous, MPT, and Rd18, respectively. In CR/VGPR patients, median TTST was 69.5, 37.7, and 39.9 months, respectively. Bortezomib (BORT) was the most common second-line treatment (Rd continuous, 59.9%; MPT, 44.6%; Rd18, 55.2%). Median time from second-line BORT to third line was longer following Rd continuous vs MPT (16.4 vs 10.6 months). No new safety signals were observed. Incidence of second primary malignancies was similar between Rd continuous and Rd18.

Conclusions

Rd continuous significantly prolonged OS and, with longer follow-up, maintained the PFS advantage vs MPT in TNE patients with NDMM. Rd continuous and Rd18 had comparable OS results. Rd continuous showed a PFS benefit vs Rd18, delaying TTST. Results reaffirm Rd continuous as a standard of care for TNE patients with NDMM.

111. Outcomes of mismatched unrelated donor transplants in Western Australia

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Aim: Evaluate the effect of donor type on outcome of allogeneic haemopoietic progenitor cell transplantation (HPCT).

Methods: Sequential patients who received first allogeneic HPCT using peripheral blood stem cells between 2004-2016 were identified from a prospectively maintained database. HLA match grade was assigned using allele-level typing at HLA A, B, C and DRBI, performed retrospectively in some cases.

Results: Patient characteristics are described in the Table. The incidence of grade II – IV acute graft versus host disease (GVHD) was high (72%) in recipients of 7/8 matched donors, compared to 8/8 matched unrelated (58%) and related donors (48%) ($p = 0.027$). This effect was even stronger for severe (grade III-IV) aGVHD ($p < 0.001$). However, no association was observed between donor type and cumulative incidence of chronic GVHD, relapse or TRM. At the median follow-up of 3 years, overall survival was 51% for the entire cohort and was strongly associated with rDRI (66% for low/intermediate vs 35% for high/very high, $p < 0.001$) and age > 45 ($p < 0.001$) but not donor type. Among recipients of 7/8 matched unrelated donors, no association was observed between the use of ATG and risk of acute or chronic GVHD, relapse or TRM. For patients who received ATG, lymphocyte count pre-ATG did not correlate with the risk of acute or chronic GVHD.

Conclusion: While use of 7/8 matched unrelated donors was associated with greater risk of severe acute GVHD, this did not result in excess chronic GVHD or mortality. Furthermore, the use of ATG for 7/8 matched transplants, currently our institutional practice, did not appear to reduce the risk of GVHD. While these observations may reflect small sample size, it is clear from this cohort that underlying disease has much greater impact than donor type on recipient outcomes.

Table: Patient characteristics	Matched related N= 147 (47%)	Matched unrelated (8/8) N = 135 (43%)	Mismatched unrelated (7/8) N = 32 (10%)
Median age, years (range)	45(17-67)	44(17-67)	39(19-62)
Disease			
Acute myeloid leukaemia or myelodysplasia	82 (56%)	73 (54%)	10 (31%)
Acute lymphoblastic leukaemia	17 (12%)	25 (19%)	5 (16%)
Non-Hodgkin lymphoma	21 (14%)	14 (10%)	9 (28%)
Hodgkin lymphoma	3 (2%)	8 (6%)	5 (16%)
Myeloma	4 (3%)	2 (1%)	0
Chronic myeloid leukaemia	5 (3%)	2 (1%)	2 (6%)
Chronic lymphocytic leukaemia	6 (4%)	7 (5%)	0
Myelofibrosis	2 (1%)	0	0
Bone marrow failure syndromes	1 (1%)	2 (1%)	0
Other	6 (4%)	2 (1%)	1 (3%)
Refined disease risk index (rDRI)			
Low	15 (10%)	19 (14%)	6 (19%)
Intermediate	60 (41%)	69 (51%)	15 (47%)
High/very high	61 (41%)	40 (30)	9 (28%)
Unavailable	11 (7%)	7 (5%)	3 (9%)
Conditioning regimen			
Myeloablative	90 (61%)	76 (56%)	19 (59%)
Reduced intensity	57 (39%)	59 (44%)	13 (41%)
Anti-thymocyte globulin (ATG) given	13 (6%)	23 (17%)	18 (56%)

112. Effect of donor age on unrelated donor haematopoietic cell transplant outcome: the Australian experience

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Aim

This study sought to determine the effect of donor age on the outcome of unrelated donor haematopoietic cell transplants (UD-HCT) in Australia, measured by engraftment, graft-versus-host disease (GvHD), overall and disease-free survival.

Method

UD-HCT recipients were selected for this study from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR) database. Patients aged 16 and above were included in the study if they underwent first allogeneic unrelated donor HCT in Australia for the indications of acute lymphoblastic leukaemia, acute myelogenous leukaemia, chronic myelogenous leukaemia or myelodysplastic syndromes between the years of 2001 and 2014 inclusive and received infusions from bone marrow or peripheral blood in adult HCT centres in Australia. The effect of donor age on outcomes was tested with other potential risk factors in a multivariate setting.

Results

A total of 1,173 UD-HCT were represented in the data. The median recipient age at transplant was 45 (range 16–70). The median donor age was 36 (range 18–59) and 866 donors (73.8%) were male. In multivariate Cox regression analysis, year of transplant 2001-2007, recipient age 40 or greater, poor risk disease, HLA match less than 6/6 and performance status at transplant less than 90 were independently significant adverse risk factors for overall survival (OS). Donor age, either as a continuous or categorical variable, was not a significant risk factor for OS or disease-free survival (DFS) in multivariate analysis.

Conclusion

Conflicting international results regarding the impact of donor age on UD-HCT outcome remain problematic. In our study, donor age was not an independent risk factor for DFS or OS. Donor age effects may have been influenced in studies by patient selection procedures at specific registries, or other factors which may be difficult to measure.

114. Multilineage dysplasia in AML with myelodysplasia-related changes confers adverse prognosis in the absence of NPM1

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Aim: Acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC) is defined by the WHO 2008 as an acute leukaemia with one or more of: multilineage dysplasia (MLD), a myelodysplastic syndrome (MDS)-related cytogenetic abnormality and/or a prior history of MDS or MDS/myeloproliferative neoplasm (1). AML-MRC has a poor prognosis, however the prognosis when due to MLD alone is unclear, with conflicting literature (2, 5). The prognosis in MLD is dependent on nucleophosmin (*NPM1*) mutations (3, 4). The 2016 WHO update excludes *NPM1* mutations from AML-MRC (Arber *et al*, 2016). This study compares survival between AML-MRC subgroups and examines the impact of *NPM1* on survival.

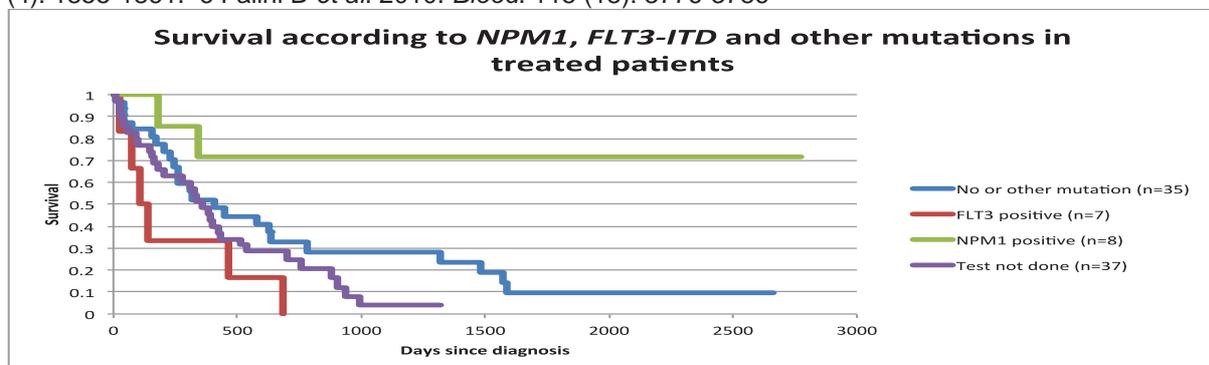
Method: This single centre retrospective observational study systematically collected demographic and diagnostic data (MLD, cytogenetics and pre-existing MDS), prognostic data including *NPM1* and *FLT3-ITD* and the treatment and outcome data from patient records using pre-specified criteria. MLD was defined as dysplasia present in at least 50% of cells in at least two cell lines; cases were reviewed by two experienced haematopathologists. Primary outcome was overall survival from diagnosis to death or end of follow up.

Results: 113 patients were diagnosed with AML-MRC from March 2008 to March 2017; 3 patients were excluded due to incomplete data. Overall survival was 17%. 23 patients were palliated; 87 treated patients were included in analysis. AML-MRC subgroups are in the legend below. Patients diagnosed by only MLD had statistically better survival compared to other subgroups ($p=0.03$). *NPM1* mutations occur at higher rates in MLD ($p<0.01$). *NPM1* mutations portend improved survival ($p=0.03$); when *NPM1* positive patients are excluded MLD loses significance. Allograft in CR1 improves survival. Graphs below.

Conclusion: *NPM1* mutations portend improved outcomes in AML-MRC. The adverse prognosis of MLD is retained only with wildtype *NPM1*, highlighting the need for mutation testing at diagnosis.

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1 Swerdlow S *et al* (Eds). 2008. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue. 2 Miesner M *et al*. 2010. *Blood*. 116 (15): 2742-2751. 3 Rozman M *et al*. 2014. *Ann Haematol*. 93: 1695:1703 4 Diaz-Beya M *et al*. 2010. *Blood*. 116 (26): 6147-6148. 5 Wandt H *et al*. 2008. *Blood*. 111 (4): 1855-1861. 6 Falini B *et al*. 2010. *Blood*. 115 (18): 3776-3786



116. Perils of tyrosine kinase inhibitors

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The increasing use of targeted agents eg. Tyrosine Kinase Inhibitors has revolutionised treatment outcomes in patients with haematological malignancies. As result, unusual off target side effects are increasingly being reported.

Here we report two such cases where clinically significant side effects were seen with Imatinib and Dasatinib.

Case 1

49 year old women of Thai origin diagnosed with Philadelphia chromosome positive Acute Lymphocytic Leukaemia (ALL) was commenced on induction chemotherapy with HyperCVAD and Imatinib.

She achieved MRD (Minimal residual disease) negativity by PCR after cycle 3A (A/ B cycles given sequentially). Unfortunately she developed worsening nausea, dysgeusia, poor oral intake and haematemesis. An urgent endoscopy showed diffuse gastropathy similar to portal hypertension gastropathy.

Imatinib has been reported in literature to cause GIVE (gastrointestinal vascular ectasia) and GAVE (gastric antral vascular ectasia) in patients treated for ALL, CML and GIST (Gastrointestinal stromal tumour). Given no other possible explanation, she was changed to Dasatinib with resolution of her symptoms.

Case 2

66 year-old Caucasian man with chronic myeloid leukaemia (CML) and severe emphysema presented with 2 month history of progressive breathlessness on treatment with Dasatinib. The chest radiograph demonstrated right-sided pleural effusion with thoracentesis showing an exudative effusion with elevated triglyceride levels (10.1mmol/L) relative to serum (1.0mmol/L) diagnostic of chylothorax.

Although pleural effusion is common, chylothoraces are infrequently reported in literature as adverse effect of Dasatinib. A diagnosis of Dasatinib-associated chylothorax was made after exclusion of other causes.

The treatment consisted of thoracentesis, dietary modification with avoidance of long chain fatty acids and replacement of Dasatinib with Nilotinib. This resulted in resolution of clinical symptoms and radiological findings with patient in Major Molecular remission (MMR) at 16 month review.

The mechanism of these side effects is unclear, but given significant morbidity and mortality treating clinicians need to be vigilant for such events.

117. A Case Report: A Cytogenetic Relapse in a Patient with Ph+ Acute Lymphoblastic Leukaemia

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Ph+ acute lymphoblastic leukaemia (ALL) is uncommon and has the worst prognosis among patients with ALL, both in children and adults. In the era of tyrosine kinase inhibitor, the outcome of treatment has significantly improved. Therefore, the genetic testing becomes critical in the management of those patients with Ph+ ALL.

We report a case of Ph+ acute lymphoblastic leukaemia patient with cytogenetic relapse when patient was in morphological and immunophenotypic remission. A 16-year-old girl was diagnosed with Ph+ (p210) pre-B cell lymphoblastic leukaemia 10 months ago. She has had standard chemotherapies and subsequently went in to morphological remission with negative MRD results on flow cytometry. PCR for bcr-abl was markedly reduced but never been completely negative. She was found 17.5% t(9;22) on conventional cytogenetic analysis during the maintenance chemotherapy whilst the bone marrow morphology was still in remission with negative MRD result on the flow cytometry.

The hypothesis is that the Philadelphia fusion genes found in the cytogenetic test may be in the myeloid cells with the possibility of pre-existing, undiagnosed chronic myeloid leukaemia with blast crisis to acute lymphoblastic leukaemia. Unfortunately, this hypothesis cannot be approved by retrospective reviewing peripheral blood film from the diagnosis. The discrepant results between morphology/flow cytometry and cytogenetic/molecular tests remain unknown.

118. Utility of metaphase FISH in determining prognostically significant cryptic KMT2A rearrangements in cytogenetically normal AML

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The identification of specific chromosomal aberrations in Acute Myeloid Leukaemia (AML) is important for the stratification of patients into appropriate prognostic groups, and dictates prescribed treatment protocols. However, a significant proportion of diagnostic bone marrow samples are reported as normal by conventional cytogenetic analysis. It is purported that a cytogenetically normal karyotype may conceal the presence of diagnostically significant chromosomal rearrangements.

Chromosomal rearrangements involving the KMT2A (MLL) gene have been associated with many different haematological malignancies, including AML. While most are identifiable by conventional cytogenetics, in some cases, cryptic rearrangements make the KMT2A involvement difficult or impossible to be detected by conventional cytogenetics. Currently, prognosis associated with KMT2A rearrangements in AML is dependent on the partner gene. Therefore, precise determination of the partner gene is important for accurate prognostic stratification.

Here, two patients <65 years and diagnosed with cytogenetically normal AML were further investigated by interphase fluorescence *in situ* hybridisation (FISH) using a panel of FISH probes targeting aberrations seen in AML: PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1 dual fusion probes, and a KMT2A break-apart probe for detection of KMT2A rearrangements. KMT2A break-apart FISH studies demonstrated a rearrangement of the KMT2A gene in both patients. Subsequent directed metaphase FISH studies indicated a cryptic insertion of 5' proximal KMT2A into chromosome 10p in one patient, representing a variant of the t(10;11)(p22;q23) rearrangement, and a cryptic insertion of 5' proximal KMT2A into chromosome 6q in the other patient, representing a variant of the t(6;11)(q27;q23) rearrangement. Both t(10;11) and t(6;11) KMT2A rearrangements are indicators of poor prognosis when compared to the standard risk group for AML, therefore changing the prognostic subgroup from intermediate to poor in these two patients. It is therefore proposed that FISH studies, including metaphase FISH, may be of clinical utility in elucidating cryptic rearrangements of prognostic significance in cytogenetically normal AML cases <65 years.

119. Acute myeloid leukaemia in the elderly - retrospective audit of current local practice and outcomes

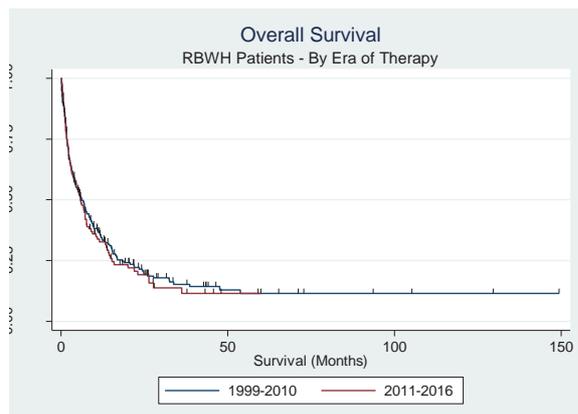
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Aims: Acute myeloid leukaemia (AML) is primarily a disease of older adults. Optimal treatment in this age group remains largely undefined. This audit aims to review local practice and outcomes of patients ≥ 60 yrs with AML presenting to our institution between 2011-2016, and compare outcomes with a historical cohort treated between 1999-2010 to determine whether changes in practice have overtly improved outcomes over this time.

Method: All patients ≥ 60 yrs presenting to our facility with newly diagnosed AML between 1/1/2011 and 31/12/16 were identified from an institutional database. Patient demographics, treatment and outcome measures were obtained retrospectively from individual patient records. Survival outcomes were assessed using Kaplan Meier method.

Results: 115 patients ≥ 60 yrs were diagnosed with AML during the time period under review. Median age was 70 years (range 60-87), with 36% female. Median overall survival (OS) was 5.85 months, with poorer outcomes seen in patients > 70 years. In total 49 patients (43%) received intensive chemotherapy, 16 (14%) azacitidine and 50 (43%) non-intensive therapies. Median survival of intensive versus non-intensively treated patients was 14.2mths versus 2.2mths respectively, $p=0.100$. Patients treated with azacitidine had similar survival as per those treated with intensive induction chemotherapy. For patients receiving intensive induction, proceeding to subsequent allogeneic transplantation appeared to improve outcomes ($n=11$; median OS 22mths versus 10.4 months intensive treatment alone. $p= 0.083$). When comparison was made to a historical cohort (1999-2010), there has been no significant improvement in overall survival over this period.



Conclusions

Our experience suggests that survival outcomes for elderly AML have not significantly improved over the last 17 yrs. Although allogeneic transplantation appears to improve outcomes, only a small minority (10%) are suitable to proceed with this therapy. This review will help benchmarking local practice to provide supportive data when making treatment decisions in the best interest of patients.

No conflict of interest to disclose

Key

Word:

Acute

Myeloid

Leukaemia

120. Low dose cytarabine and thioguanine for acute myeloid leukaemia: The Sir Charles Gairdner Hospital experience

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Aim

Acute Myeloid Leukaemia (AML) is a disease associated with poor prognosis particularly in elderly and unfit patients. We adopted the Royal North Shore protocol (RNS) consisting of subcutaneous low dose cytarabine and oral thioguanine in 2012 based on promising results in patients deemed unsuitable for intensive therapies¹. The aim of this study was to assess our institutional outcomes.

Method

We identified all cases treated at our hospital through a retrospective chart, database and pharmacy review from adoption of this protocol in April 2012 to July 2017.

Results

38 patients were identified. The median age of patients treated was 76.5 years. [range 50-87] with 84% >65 years old. There was a male predominance. 15 (40%) had de novo AML; 16 (42%) had AML transformed from myelodysplasia and 7 (18%) had therapy related AML. By karyotyping 26 (69%) had intermediate risk; 8 (22%) had adverse risk, 1 (3%) favourable risk and 2 (6%) were unknown. 23 (61%) had RNS therapy as first line, 11 (29%) as second line and 4 (10%) third line or beyond. The overall response rate was 7/38 (18%) consisting of 5 (13%) complete remission (CR) and 2 (5%) complete remission with incomplete recovery (CRi). The median progression free survival of patients in CR was 54 weeks and 6 weeks for those who achieved CRi. The median overall survival of the entire cohort was 13 weeks [range 1-92]. Four of the six patients who achieved CR/CRi died of AML.

Conclusion

AML in elderly or unfit patients is associated with a dismal prognosis. Patients who achieve a CR with adequate count recovery can have extended remissions with subcutaneous low dose cytarabine and oral thioguanine. Novel therapies are urgently required.

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1. Arthur C, Wong C, Aubrey B, Liu E, Stevenson W, Soo G, et al. Progress Findings On a Novel Treatment Strategy Using Prolonged, Low-Dose Cytarabine and Thioguanine in Combination with Peg-Filgrastim for Acute Myeloid Leukemia in Elderly Patients. *Blood*. 2012;120(21):3612.

121. Clinical utility of sequential G-band to FISH testing to elucidate chromosomal abnormalities in paediatric leukaemias

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Aim

To demonstrate improved cytogenetic prognostication using sequential metaphase G-band to FISH testing in paediatric acute leukaemias with complex/variant cytogenetics.

Methods

A combination of oncology whole chromosome paints (WCP), locus specific indicator (LSI) probes and break-apart (BAP) probes were used to further investigate karyotypic abnormalities of three paediatric acute leukaemia cases of different cellular lineages. Metaphase G-banded chromosomes were sequentially hybridized for FISH analysis.

Results

Case 1: A child with B-cell acute lymphoblastic leukaemia (ALL).

G-band analysis showed a simple dicentric abnormality dic(Y;20). However sequential metaphase CRLF2 BAP and interphase IGH@ BAP FISH testing demonstrated a variant CRLF2-IGH@ fusion typically resulting in overexpression of CRLF2. This kinase alteration is encompassed within the recently defined Philadelphia chromosome-like ALL group, which tends to a poorer prognosis.

Case 2: A child with T-cell ALL.

G-band analysis detected a translocation t(7;9) in 2 cells and the same abnormality in 5 cells of an evolved tetraploid sideline clone. Sequential T-cell receptor beta (TCRB) BAP FISH testing confirmed this rearrangement. TCRB rearrangements are consistent with T-cell ALL and the G-band breakpoints favoured a TCRB-TAL2 rearrangement seen in <1% of T-ALL.

Case 3: A child with acute myeloid leukaemia (AML).

A combination of 5q platelet derived growth factor receptor beta (PDGFRB), LSI probes and WCP's were used to elucidate a complex rearrangement between two chromosome 5's and one chromosome 6 in an AML patient. This finding is rare in AML and FISH confirmed no apparent deletion of 5q which most likely excludes this patient from the poor prognosis associated with AML and del(5q).

Conclusion

Sequential metaphase G-band to FISH testing in addition to G-band analysis elucidates variant abnormalities in complex paediatric leukaemic karyotypes to provide more accurate identification of cytogenetic prognostic markers.

122. Management of pegasparginase-induced hepatotoxicity with combination of oral and intravenous L-carnitine and vitamin B complex

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Introduction: The incidence of pegasparginase-induced hepatotoxicity in adults with acute lymphoblastic leukaemia (ALL) has been reported to be up to 60%. Prompt management is important to ensure further chemotherapy is not delayed or contraindicated. Hepatotoxicity is thought to be due to depletion of L-asparagine in the liver, resulting in impairment of mitochondrial β -oxidation and accumulation of unoxidised fatty acids. Vitamin B complex and L-carnitine are important mitochondrial cofactors; there have been several case reports describing their usage for the treatment of asparaginase-induced hepatotoxicity.

Case Presentation: A 46-year-old male received pegasparginase 2500 International Units/m² two weeks post FLAG induction chemotherapy for relapsed ALL. Baseline liver function tests (LFTs) were slightly elevated on the day of administration, with ALT 60 IU/L, AST 23 IU/L, GGT 75 IU/L, ALP 173 IU/L and bilirubin 15 micromol/L (Figure 1). Following pegasparginase treatment his LFTs rose steadily, with a bilirubin of 45 micromol/L at day 10, before peaking at 292 micromol/L on day 22.

On day 23, oral acetyl-L-carnitine 1g daily and vitamin B complex two tablets daily were commenced. A liver biopsy on day 25 revealed severe macrovesicular hepatosteatosis. From day 30, the patient was administered intravenous (IV) L-carnitine 4g daily (approximately 50mg/kg). On day 41, given significant improvement in LFTs, IV L-carnitine treatment was discontinued. A total of 11 IV doses were administered over the 12-day treatment period. The patient continued oral acetyl-L-carnitine and vitamin B complex until day 70 by which time his LFTs had further improved, with ALT 72 IU/L, AST 41 IU/L, GGT 124 IU/L, ALP 86 IU/L and bilirubin of 7 micromol/L. The patient was able to proceed with further therapy for ALL.

Conclusion: This is the first reported case in Australia to use a combination of oral and intravenous L-carnitine and vitamin B complex to successfully treat pegasparginase-induced hepatotoxicity.

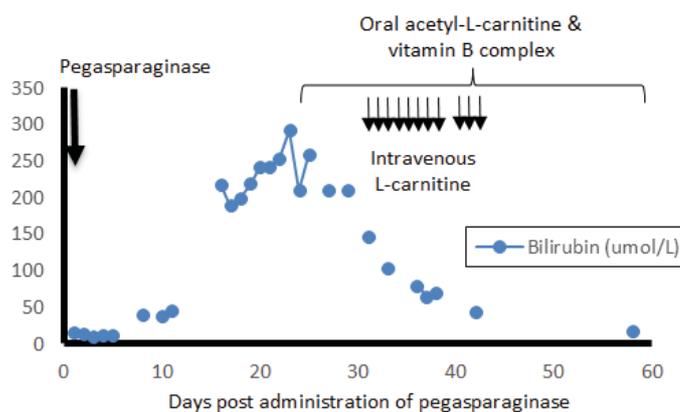


Figure 1. Bilirubin values corresponding with administration of pegasparginase, L-carnitine and vitamin B complex

123. Outcomes in older patients with acute myeloid leukaemia (AML)

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Aim

AML is a cancer of older persons (mean age 64.2yrs). At our centre, patients aged 60-69 receive induction chemotherapy as standard of care. Older patients (≥ 70 -75) are selected if they have a good performance status, minimal co-morbidities and according to physician preference. This audit seeks to compare the outcomes of older AML patients.

Method

This was a single centre retrospective chart review on all (n=46) patients ≥ 60 yrs that underwent induction chemotherapy for AML between June 2009 and June 2016, with minimum follow up of 12m (or until death). Patients were stratified according to age (60-69, n=29 and ≥ 70 , n=17) and intermediate versus poor cytogenetics.

Result

Six patients received an allograft (all with intermediate cytogenetics). Their median OS and DFS was 24.3m and 23.4m respectively. In non-transplanted patients with intermediate cytogenetics, those 60-69 (n=15), had comparable outcomes to those ≥ 70 (n=12). OS 6.8m (0.10-14.5) vs 10.2m (3.8-43.5), PFS 5.0m vs 6.9m and DFS (=CR1) 8.1m vs 6.0m, with CR1 occurrence of 53% vs 91%. In patients with poor cytogenetics, those 60-69 (n=8) had comparable survival outcomes to those ≥ 70 (n=5). OS 4.5m (1.4-16.5) vs 8.3m (2.8-10.2), PFS 2.7m vs 1.3m and DFS (=CR1) 8.0m vs 6.5m. Those ≥ 70 had inferior CR1 rate of 40% vs 75% and the highest LOS for induction 1 with 80% >30-day admission. Across all groups, TRM was highest in the 60-69 group (5 vs 0), as were ICU admissions (4 vs 1).

Conclusion

Selected patients aged 70-75 can have similar outcomes to their younger counterparts when receiving induction therapy, with no significant increase in TRM or ICU admission. This is most pronounced in those with intermediate cytogenetics. Patients aged 70-75 with poor risk cytogenetics have a lower CR rate and higher LOS compared to their younger counterparts. Options other than induction therapy should also be considered.

124. Lipegfilgrastim is an adequate alternative for supporting neutrophil recovery in acute leukaemia after induction chemotherapy

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Aim

Patients with acute leukaemia (AL) are typically neutropenic for prolonged periods after induction chemotherapy. Granulocyte colony stimulating factors (GCSFs) are commonly used to reduce this duration of neutropenia. Lipegfilgrastim is a newer long acting glycol-pegylated GCSF that is registered for use in Australia. We sought to establish the adequacy of its use in AL patients undergoing induction.

Method

We retrospectively analysed the medical records for all patients diagnosed with AL that received lipegfilgrastim in our metropolitan haematology unit. Patient demographics, disease, chemotherapy, outcome and dates of neutrophil recovery were recorded. Date of neutrophil recovery was defined as an absolute neutrophil count greater than $0.5 \times 10^9/L$. We then compared results with two other conveniently selected cohorts of patients that received either pegfilgrastim or filgrastim post induction for acute myeloid leukaemia (AML) only.

Results

23 patients (18 AML, 5 acute lymphoblastic leukaemia (ALL)) underwent 24 induction cycles of chemotherapy and received lipegfilgrastim at least 24 hours after completion of the last dose of chemotherapy. 11 of the 18 AML patients and 4 of the 5 ALL patients achieved a complete remission after induction. In the 11 AML patients that achieved remission after induction, the median time to recovery was 21 days [17-27] and the median time to discharge was 23 days [18-38]. This compares with 11 AML patients from the filgrastim cohort (median 21 days to recovery [19-25], 22 to discharge [18-27]) and 11 AML patients from the pegfilgrastim cohort (median 21 days to recovery [17-27], 21 to discharge [18-32]).

Conclusion

Lipegfilgrastim is an adequate GCSF for use in patients with acute leukaemia, demonstrating a similar time to neutrophil recovery compared with our own experience and with that of published data of alternative GCSFs.

125. Clinical Features of Acute Myeloid Leukemia (AML) patients in Dharmais Cancer Hospital: A Descriptive Study

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Objective: To describe the clinical features of acute myeloid leukemia (AML) patients, as a factor that could influence the patients' prognosis and survival.

Methods: A total of 28 from 41 new AML patients admitted during 2016 in Dharmais Cancer Center Hospital (Jakarta, Indonesia) were included as the participants in this cohort retrospective study. The clinical features measured including age, ECOG performance status, prior hematological disease, therapy-related AML, serum LDH, WBC count, extramedullary involvement, and infection. These information were collected from medical record. For analysis, these clinical features were categorised into favorable, intermediate, or unfavorable.

Result: Among all of participants, the proportion of male and female were equal (50%), with average age of 48.03 years. This average age was significantly lower (favorable) than the one of AML patients in USA. It was shown also that 18 of 28 subjects (64%) had favorable ECOG performance status. 27 subjects (96%) had no prior hematological disease (favorable). All the subjects were the novo AML (favorable). 16 subjects (57%) had favorable WBC count. 18 subjects (64%) had no extramedullary involvement (favorable). 22 subjects (78,5%) had no infection when diagnosis was established (favorable). On the other hand, 15 subjects (53.5%) had higher LDH serum (unfavorable).

Conclusion: The characteristics of AML patients in this study showed that they had more favorable clinical features. This condition could bring hope for improvement in patients' prognosis and survival, even though these clinical features were not the only factor that could influence patients' prognosis.

Key words: acute myeloid leukemia (AML), clinical features, prognosis

126. A case of Philadelphia chromosome positive acute myeloid leukaemia

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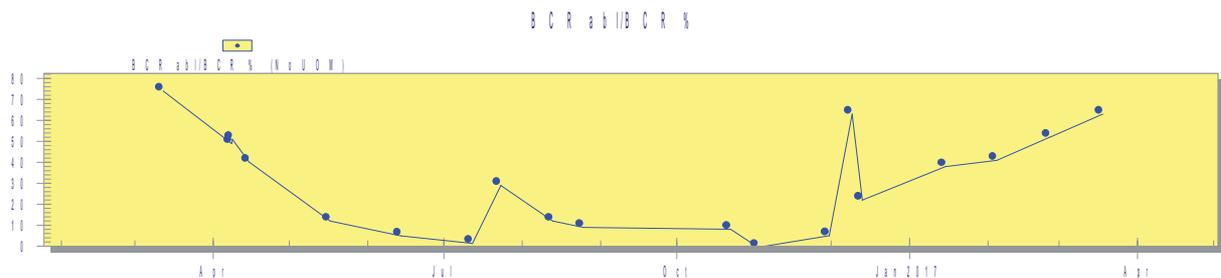
Background: Philadelphia chromosome positive acute myeloid leukaemia is rare. We describe our experience of treating a patient with this condition, distinguishing it from chronic myeloid leukaemia in blast crisis and a review of the literature.

Case presentation: We describe the case of Mr Smith, a previously fit and well gentleman who presented with symptomatic anaemia. He was pancytopenic at time of diagnosis, March 2016, with a peripheral blast count of 1.08 (25-30%); qPCR BCR-ABL 75% (major breakpoint). He had a stormy course with primary refractory disease after induction chemotherapy and dasatinib, followed by brief morphological remission (qPCR BCR-ABL 5.9%). He relapsed a month later requiring further chemotherapy. He was in remission again in August 2016 and underwent a matched unrelated donor allogeneic transplant in September 2016. Following transplantation, he had frank relapse in December 2016 and was commenced on azacitidine. Dasatinib was changed to ponatinib due to gastro-intestinal side effects. Unfortunately, he had progressive disease and died in May 2017.

Discussion: The Philadelphia chromosome is the genetic abnormality characteristic of chronic myeloid leukaemia (CML) and is present in 30% of adults with B cell acute lymphoblastic leukaemia. It is a rare abnormality in acute myeloid leukaemia with a described incidence of 0.5-3%. The main differential is that of CML-blast crisis with some identified clinical and cytogenetic/molecular differences.

The literature describes patients treated with combinations of chemotherapy, tyrosine kinase inhibitors (TKI, primarily imatinib in that era) and allogeneic transplantation. Median survival was 18 months (range 6-71 months), survival without a TKI was 7 months.

Conclusion: Philadelphia chromosome positive acute myeloid leukaemia remains a rare entity with significantly improved results when treated with a combination of chemotherapy and tyrosine kinase inhibitors. Allogeneic transplantation should be considered in eligible patients.



No conflict of interest to disclose.

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127. Core Binding Factor Acute Myeloid Leukaemia: An audit of patient outcomes over 20 years.

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Introduction

Core Binding Factor (CBF +) Acute Myeloid Leukaemia (AML) is an uncommon subtype of AML characterised by recurrent cytogenetic abnormalities [t(8;21), inv(16), t(16;16)] and is seen in up to 15% of cases of de novo AML. It is associated with a relatively favourable prognosis when treated with cytarabine based chemotherapy alone, although up to 30-40% of patients eventually relapse.

Method

A retrospective review at a single centre of all cases of CBF+ AML diagnosed between 1/1/1997 to 31/5/2017 at The Canberra Hospital was conducted. 16 cases were identified with 6 cases of t(8;21), 10 of inv(16) and no cases of t(16;16).

The research was approved by the jurisdictional Ethics Committee. Medical records were interrogated for acknowledged prognostic factors (age, additional genetic abnormalities, WBC at diagnosis, MRD persistence post treatment) as well as treatment protocol, frequency and technique of genomic monitoring and patient outcomes (CR, PFS, OS).

Results

Of the patients with t(8;21), 2 of 6 cases had relapsed and proceeded to Allogeneic bone marrow transplantation after salvage chemotherapy. The 4 patients in remission continue to have molecular monitoring of their AML. Of the 10 patients with inv(16) AML, 3 had relapsed and 1 of the relapsed patients was monitored by molecular tests.

Conclusion

Our audit has confirmed that in our centre, we see a similarly favourable prognosis and lower relapse rate in CBF + AML compared with other AML subtypes. With molecular monitoring, ongoing complete remission can be confirmed, and early molecular relapse can be identified and alternative treatment options offered earlier to suitable patients.

Clinical significance

Molecular monitoring in CBF AML improves patient management.

128. Two cases of differentiation syndrome chorioretinopathy in acute promyelocytic leukaemia patients treated with differentiation therapy

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Aim

To describe two cases of differentiation syndrome (DS) presenting with ocular manifestations in patients with acute promyelocytic leukaemia treated with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) differentiation therapy.

Method

This observational case series identifies two patients at Gold Coast University Hospital, a single tertiary institution, diagnosed with DS with associated ophthalmic involvement.

Results

Both patients reported bilateral reduction in visual acuity at days fourteen and ten respectively following initiation of differentiation therapy in addition to developing other systemic manifestations of DS. Both patients received the same APLM4 chemotherapeutic regimen including an anthracycline and both ATRA and ATO as well as ten days of routine DS prophylaxis with oral prednisolone¹. Case 1 presented with bilateral angle closure and multifocal areas of sub-retinal fluid. Case 2 presented with similar characteristic retinal findings on fundoscopy and optical coherence tomography. Case 1 was managed with a prolongation of corticosteroids in addition to temporary cessation of ATRA and ATO for presumed DS. This diagnosis in case 2 was made on review of her case after induction therapy was complete and no specific DS management was implemented. Both experienced rapid improvement in visual symptoms and marked resolution of the sub-retinal fluid within seven to fourteen days of onset with excellent long-term visual outcome. Both patients were in molecular remission after induction therapy and went on to receive ATRA and ATO maintenance without steroid cover and without visual problems.

Conclusion

Ocular manifestations of DS have been only recently recognised². Timely ophthalmic assessment of patients presenting with visual symptoms in the context of differentiation therapy is critical for diagnosis and ensuring optimal ophthalmic outcomes. Once the incidence and nature of ophthalmic involvement in DS is more fully appreciated, the role and timing of ophthalmic examination in patients receiving differentiation therapy can be evaluated.

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129. Anthracycline intensification, combined with intermediate-dose cytarabine in consolidation well tolerated in older AML patients

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Aim: The HOVON group data demonstrated the benefit of anthracycline intensification during induction in patients aged 60-65 years, while the ALLG AML M12 study demonstrated the value of anthracycline intensification during consolidation in younger adults. We have implemented a novel combination of intensified anthracycline in combination with infusional cytarabine during induction, with intermediate-dose cytarabine during consolidation.

Method: A retrospective study was done on 76 patients aged 55 years or greater with newly diagnosed AML between January 2010 to June 2016 at the Alfred Hospital, Melbourne. All received 7+3 induction (cytarabine continuous infusion 100mg/m²/day days 1-7, and idarubicin 12mg/m²/day days 1-3), with a planned consolidation with 2 cycles of IDAC-2 (cytarabine 1000 mg/m² twice daily Day 1, 3, 5, and idarubicin 12 mg/m²/day Day 1 – 2). Outcomes were assessed according to the Cheson criteria.

Results: 76 patients, with a median age of 62 years (range 55.4 – 70.6 years) received 7+3 induction with a median OS of 590 (range 6 – 1996) days and the CR with CRi was 52 patients (68.4%). The EFS median is 109 days (range 6 – 1988) and the RFS median is 314 days (range 4 – 1947). There were 9 treatment-related deaths (11.8%). Of 41 patients with CMR after induction, 29 patients (70.7%) received IDAC-2, with 18 (41.5%) receiving two consolidation cycles. No IDAC-2 treatment-related deaths occurred. 8 patients (27.6%) receiving IDAC+2 proceeded to an allogeneic SCT. In all IDAC-2 cycles, the median time to neutrophil recovery was 26 days, platelet recovery 32 days, and the ICU admission rate was 12.8%. 18 patients (62.1%) receiving IDAC-2 suffered disease relapse. In patients aged 60-65 years, the remission and survival rates were similar to the HOVON group data¹ (table 1).

Conclusion: Anthracycline intensification was well tolerated in older AML patients. Despite this intensive post-remission therapy approach, rates of disease relapse were high, highlighting the need for novel therapeutic approaches.

Table 1 – Comparison with Lowenberg et al NEJM 2009 for patients 60-65

Parameter	7+3 / IDAC+2 (n=30) (intention to treat population)	HOVON/SAKK escalated DNR (n=145)
Complete Remission	77%	73%
EFS at 2-years	15%	29%
DFS at 2-years	18%	39%
OS at 2-years	46%	38%
Early death (30-days)	3%	12% in overall cohort

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No conflict of interest to disclose

130. Clinical outcomes of Acute Myeloid Leukemia in Western Australia: a retrospective multicentre study

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Aim

The study was performed to assess the effect of karyotype and molecular alleles of FLT3 ITD and NPM1 and other clinical parameters on overall survival outcomes in the WA cohort.

Method

A retrospective multicentre analysis was undertaken of all consecutive adult AML patients (n=599) presenting to tertiary hospitals in WA from 2008 to 2016. Contribution of molecular, cytogenetic factors and effect of treatment on survival was assessed. We used Kaplan-Meier method to assess overall survival (OS), p value <0.05.

Result

In the entire cohort, gender had no impact on survival (males (n=328) versus females (n=271) of 42.7 versus 34.7 months, p=0.3) while age <65 years (n=336) had a significant survival advantage (50.1 versus 16.7 months, p<0.0001). Primary versus secondary AML significantly impacted survival (43 versus 18 months, n=429 versus 170, p<0.001) similarly intensive chemotherapy and allogeneic transplantation (p<0.001) improved survival. Of the patients that received intensive chemotherapy (n=381), karyotype significantly influenced survival with favourable, intermediate and adverse cytogenetics resulting in median OS of 76.6, 44.4 and 27.7 months respectively (p=0.0001). Within the intermediate risk group, there was no association between FLT3-ITD or NPM1 status and overall survival (p=0.3) although survival was improved in patients who underwent allogeneic transplantation, regardless of molecular status (p=0.001). In patients >65 years old, survival was associated with treatment. The mean OS after intensive chemotherapy (n=79) was 25.1 months, after azacitidine (n=19) was 17.03 months, and thioguanine and cytarabine (n= 17) was 7.3 months (p=0.001).

Conclusion

Age, cytogenetics and denovo AML and treatment significantly influenced outcomes in our AML patients. Survival rates have improved which may reflect better supportive care, aggressive chemotherapy and allogeneic stem cell transplantation.

131. Outcomes and Healthcare utilization in older patients with Acute Myeloid Leukaemia

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Aims:

We aimed to characterise the health care use and outcomes for older patients age ≥ 60 years diagnosed with acute myeloid leukaemia (AML) in one local area health network in South Australia.

Methods:

Consecutive patients diagnosed with AML between January 2005 – December 2015 at two hospitals were retrospectively identified. Patient medical records were reviewed for outcomes, health care use and palliative care referral. ECOG and Charlson comorbidity index were calculated retrospectively.

Results:

AML was diagnosed in 209 patients, and 186 (89%) were dead at time of analysis. The median age at diagnosis was 69 (range 60-91). 157 (75%) patients had de novo AML. 55% received intensive chemotherapy (IC) and of these 17% underwent stem cell transplant (SCT). Median survival was 6 months for the entire cohort, 10 months for patients who received IC, 16 months for SCT, 17 months for single-agent therapy and 1 month for best supportive care. Only 79 (38%) and 45 (22%) of the total cohort were alive at one and two years respectively. Of patients who died, 60% were referred to palliative care with 30% receiving end of life care within a hospice or local health care setting. Cause of death was mainly due to progression of AML. Within 30 days of death, 10% were in ICU, 62% hospitalised and 49% died in hospital. Median time from palliative care referral to death was 21 days. Overall the total cohort spent median time of 35% of survival time in either hospital or outpatient settings. Less country patients died in hospital than their metropolitan counterparts (38 vs 61).

Conclusion:

This study confirms the poor 2 year overall survival of older AML patients. There is an ongoing lack of early palliative care referrals. There also remains a more intensive utilisation of hospital services compared with outpatient settings

132. Optimisation of haematopoietic differentiation of human induced pluripotent stem cells to study aberrant haematopoiesis

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Rationale: Direct comparison between induced pluripotent stem cell (iPSC) haematopoietic differentiation methods is essential for informed selection of the optimum method for a particular endpoint. Yet despite over 100 publications and increasing numbers of new methods patented each year, there are virtually no published comparisons of methods.

Aim: The aim was to compare published methods, to identify the optimum method for haematopoietic cell production, relative cost, and utility to study aberrant haematopoiesis.

Method: Four methods were selected that are free of feeder layers and serum: two embryoid body and two monolayer, each with simple versus multistep differentiation. Various iPSC lines and independent replicates were used to determine haematopoietic differentiation efficiency, haematopoietic progenitor cell production and function, relative cost, and the capability to recapitulate aberrant haematopoiesis of the originating human subjects. Statistical analysis included ordinary one-way analysis of variance and unpaired t-tests.

Results: The monolayer multistep differentiation (2D-multistep) method resulted in greater haematopoietic differentiation efficiency, higher CD34+ progenitor cell production, both in purity and absolute numbers ($p < 0.0001$), and higher numbers of functional haematopoietic progenitors in colony forming unit (CFU) assays ($p < 0.0001$). Regarding capacity to recapitulated aberrant haematopoiesis, only the 2D-multistep method showed expected increased frequency of all Down Syndrome-derived CFU types ($p = 0.0079$). The 2D multistep method also demonstrated decreased total CFU ($p = 0.0296$) and burst forming unit-erythroid (BFU-E) ($p = 0.0341$) from β -thalassaemia-derived iPSC, consistent with previous reports. Haemoglobin gene expression showed all methods express similar amounts of α -globin mRNA, with minimal detectable β -globin.

Conclusion: The 2D-multistep method resulted in the greatest production of CD34+ haematopoietic progenitors, was the most cost effective means of producing CD34+ cells and CFU, and had the capacity to recapitulate different forms of aberrant haematopoiesis from patient-derived iPSC.

133. Targeting Blastic Plasmacytoid Dendritic Cell Neoplasm with Venetoclax and low-dose cytarabine: a case report

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Introduction

Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) is a rare and aggressive haematological malignancy commonly involving the skin, bone marrow and lymph nodes. There is no standard of care; ALL, AML or lymphoma-directed chemotherapy regimens have been tried, however prognosis remains poor with conventional therapy. Novel approaches are required, especially for older patients unlikely to tolerate intensive treatment.

BCL-2 is overexpressed in BPDCN, and transient responses have been reported with the BCL-2 inhibitor Venetoclax (Montero *et al*, Cancer Disc 2016). Venetoclax combined with low-dose cytarabine (LDAC) is well-tolerated and has promising activity in elderly patients with AML (Wei *et al*, EHA 2017), but Venetoclax/LDAC has not been described in BPDCN.

Case description

A 79 year old man presented with 12 months of progressive cutaneous lesions. Examination revealed >50 erythematous or violaceous patches and nodules involving trunk and limbs, with no lymphadenopathy or hepatosplenomegaly. Skin biopsies were consistent with BPDCN, showing CD4, CD45, CD56, CD123 expression, with Ki67 70%. BCL-2 was strongly positive. Bone marrow was not involved. PET identified multiple moderately avid subcutaneous nodules.

The patient received a novel treatment approach using LDAC 20mg/m² daily for 10 days and venetoclax 100/ 200/ 400mg (day 1, 2, 3), followed by 100mg day 4-14 of a 28 day cycle, delivered predominantly as an outpatient. This was combined with posaconazole from day 4 to potentiate venetoclax levels through CYP3A4 inhibition (Agarwal *et al*, Clin. Ther. 2017).

Within 10 days there was marked lesion regression. The patient experienced therapy-related grade 4 neutropenia lasting 10 days; no other complications were observed. The patient remains on therapy, with confirmed metabolic response after 3 cycles.

Discussion

This case highlights the overexpression of BCL-2 in BPDCN, the rapid and well-tolerated response to Venetoclax/LDAC, and the potential of this low-intensity treatment in a disease with high unmet need. Further clinical studies are warranted.

134. Acute monoblastic leukaemia presenting with fulminant marrow haemophagocytosis.

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Introduction

Adult haemophagocytosis syndromes results from excessive inflammatory cytokine release with subsequent macrophage hyper-activation in response to various stimuli, including infections, autoimmune diseases and more uncommonly lymphoid neoplasms. The present case reports a very rare presentation of adult acute monoblastic leukaemia (AML) presenting with fulminant marrow haemophagocytic histiocytosis in the presence of clonal karyotypic abnormality.

Case

A 54 year old previously well lady presented with malaise, lethargy and myalgia. She was found to be febrile at presentation. Examination revealed petechiae and ecchymoses in the absence of lymphadenopathy or hepatosplenomegaly. Initial blood count revealed mild normocytic anaemia and neutropenia and severe thrombocytopenia with platelet count of $8 \times 10^9/L$. Iron studies demonstrated ferritin of 2929 microgram/L. Blood film demonstrated microangiopathic haemolysis with occasional hypogranular neutrophil and infrequent blast.

Summary

Urgent bone marrow aspirate and trephine demonstrated hypercellular marrow replaced by morphologically normal histiocytes with marked haemophagocytic activity. There was near absence of normal haematopoiesis. Flow cytometry did not demonstrate underlying abnormal myeloblast or lymphoid population. A vigorous investigation for other underlying infective, autoimmune or malignant condition failed to demonstrate a secondary cause for the histiocytosis.

Due to these findings a trial of immunosuppression with immunoglobulin, cyclosporin and corticosteroids was implemented. Repeat marrow after 10 days of therapy demonstrated acute monoblastic leukaemia confirmed by flow cytometry and immunophenotyping. Cytogenetics on initial marrow subsequently became available demonstrating trisomy 6 in 6/20 cells.

Discussion:

This is a very rare presentation of AML, the morphological and flow cytometry features of which were masked by fulminant haemophagocytic activity. There was eventual confirmation of clonal karyotypic abnormality on initial marrow cytogenetics. Immunosuppression reduced cytokine driven histiocytic activity with subsequent repeat bone marrow biopsy confirming the presence of underlying myeloid leukaemia.

135. Ph+ ALL in the tyrosine kinase inhibitor era-improved outcomes? The experience of a single institution.

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Introduction: The Philadelphia chromosome (Ph⁺) is the most frequent chromosomal anomaly found in adult acute lymphoblastic leukaemia (ALL) in those over 45y and is reported in 20-30% of cases¹. Ph+ ALL is associated with poor prognosis. The application of tyrosine kinase inhibitors to the treatment of Ph+ ALL appears to have resulted in improved remission rates however longer-term leukaemia free and overall survival for many, remains less than optimal.

Aims: To compare treatment outcomes in our institution in adults with Ph+ ALL during the tyrosine kinase era with historic controls.

Methods: All cases of Ph+ ALL who underwent treatment between 2002 and 2016 were identified retrospectively using a disease coding electronic search method. Patient demographics and patient and treatment specific variables were collated. Because of a lack of reliable data pertaining to Ph+ ALL patients at our institution prior to 2000, our results are compared with the results seen in a highly cited international study conducted at the end of the pre-TKI era².

Results:

- 14 patients with Ph+ ALL were identified with a median age of 52.5y (25-83y).
- 79% were male (n=11)
- Median WCC at diagnosis was $25.15 \times 10^9/L$ (2.3-132.4).
- Chemotherapy regimens were variable but all patients received imatinib as part of first line induction therapy concurrent with cytotoxic therapy.
- CR rate at D+30 was 86 % (molecular/cytogenetic CR 43%).
- Median BCR ABL at diagnosis and 6 months was 37.5% and 0%
- 6 patients received allogeneic SCT.
- Median LFS was 20.4 months (0-91.4 months).
- Median overall survival (OS) was 19.7 months (5.4-100.2 months)
- 2-year LFS = 36%
- 2-year OS = 50%

Conclusion: Our study found improved LFS and OS when compared to long term survival rates of less than 20% in the pre-TKI era². This is comparable to other international assessments of outcomes with both first and second generation TKIs^{3,4}

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136. FISH to go

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Aim:

To establish a rapid FISH method (<1 hour total time) for acute and chronic leukaemia diagnosis to enable combined morphology and FISH reporting on the same day as collection at the ICPMR, NSW Health Pathology.

Method: Peripheral blood or bone marrow smears from newly presented leukaemia patients at the ICPMR were fixed and aged and FISH testing performed.

The Vysis DNA FISH probe was prepared with Vysis IntelliFISH hybridization buffer (Abbott Molecular) and smears and probe were co-denatured on a ThermoBrite® slide denaturation/hybridization system at 80°C for 2 minutes. The samples were tested with probes for the leukaemia gene rearrangements PML/RARA, RUNX1T1/RUNX1, CBFB, KMT2A and BCR/ABL1. Duplicate smears were hybridized at 37°C for either 10 minutes or 1 hour. Analysis was performed using the BioView Duet™ software. Results of the 10 minute hybridization time were compared to the recommended hybridization time using the IntelliFISH buffer of 1 hour (range 1-18 hours), as well as to the genetic findings from an external laboratory. Duplicate smears were compared for signal strength, hybridization quality and the percentage of cells displaying the expected abnormal signal pattern.

Results:

Reducing the hybridization time to 10 minutes had no detrimental effect on the quality of the FISH signals and will enable same day integrated reporting of morphology and FISH genetic results into the one report.

Conclusion: "FISH to go" will enable the same day reporting of the genetic rearrangements of leukaemia patients to be achieved in a combined morphology and genetic report. This will lead to quicker classification and diagnosis of haematological malignancies, as correlation of the morphology and genetic results occurs on the day of collection, and therefore faster access to targeted therapies for patients.

137. Comparison of public vs private autologous stem cell transplantation outcomes for myeloma in two large tertiary hospitals in Brisbane, Australia

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Background

Myeloma is managed in both the private and public sectors and both offer similar treatment options including autologous stem cell transplantation (ASCT). For a number of potential reasons, decision on which patients to transplant could differ between the two yet this has never been studied in Australasia.

Aims

To compare patient characteristics and major outcomes for ASCT performed for myeloma in a large private versus a large public hospital in Brisbane.

Methods

We searched ASCT databases at each hospital and identified all patients who received first transplant for myeloma between 2006-2016. We compared gender and age at transplant using a two-tailed t test. We then compared the pre-specified outcomes of overall survival and relapse-free survival using Kaplan Meier estimates of survival for each site and performed log-rank comparison of the two. We also compared the risk of transplant-related mortality, defined as mortality within first 100 days, using a two-tailed t-test.

Results

251 patients were identified constituting 4.6% of all ASCTs performed for myeloma in Australia and New Zealand between 2006-2016¹.

- There was no difference in patient gender
- Privately treated patients were on average 2.98 [0.81–5.15] years older.
- There was no difference in the major outcomes of transplant-related mortality (0% private vs 4% public, p=0.08), overall survival (mean 78.8 months private vs 79.0 months public, p=0.24 (Fig1)) or relapse-free survival (mean 53.7 months private vs 63.2 months public, p=0.27 (Fig2))

Conditioning regimens and supportive care were similar between the groups

Conclusions

There was a significantly older cohort of patients receiving ASCT in the private setting compared to the public however treatment outcomes were not different. Our findings should offer reassurance to physicians and to patients undergoing ASCT in this country and support the assumption of equivalence of care between private and public medicine in Australia.

138. A challenging mobilisation and bloodless stem cell transplant

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Aim

We describe a case report of a blood-free BEAM Autologous Stem Cell Transplant despite substantial deviation from the intended mobilisation protocol.

Case Report

A patient with relapsed Stage 2A Hodgkin's Lymphoma was planned for BEAM Autograft following salvage ICE chemotherapy. The transplant was complicated by several issues. There was accidental omission of the first 6 days of GCSF injections post chemotherapy. This resulted in a lower than expected CD34+ count during harvest, requiring extension of apheresis to 3 days to obtain the target of $5 \times 10^6/\text{kg}$. The patient was a Jehovah's Witness thus declining blood products.

Strategies were implemented to maximize patient safety and ensure a coordinated multi-disciplinary approach. Education sessions were carried out with the patient and their partner by the CNCs and Haematologist. Medical and nursing staff were educated by the Haematologist and the Jehovah's Witness hospital liaison team prior to transplant and the patient's advanced care directive regarding blood product transfusion was clearly documented. To speed marrow recovery a higher collection target of $5 \times 10^6/\text{kg}$ CD34+ (rather than $3 \times 10^6/\text{kg}$) was set and a total cell dose of $5.62 \times 10^6/\text{kg}$ was collected by day 8 of GCSF. The full dose was reinfused. 3 times a week blood collection using paediatric tubes and mandatory medical officer review prior to collection of blood cultures was implemented to minimise excessive blood loss.

The patient underwent BEAM autograft, experiencing grade 3 febrile neutropenia, grade 2 diarrhoea and grade 2 mucositis (as per CTCAE grading). Prophylactic tranexamic acid was used when platelet count $< 20 \times 10^9/\text{L}$, and early commencement of GCSF was employed to hasten engraftment. The patient achieved neutrophil engraftment on day +9, platelet engraftment on day +11, and was discharged on day +15 without further complications.

Conclusion

In selected patients with good bone marrow reserve, it is feasible to undergo autologous stem cell transplantation without blood product support.

139. Successful Assisted Fertility after Allograft for Hodgkin Lymphoma

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Aim

To review fertility outcomes for patients having undergone successful allogeneic transplantation for advanced Hodgkin Lymphoma in Western Australia.

Method

We reviewed the database for Allogeneic transplants undertaken for Hodgkin Lymphoma in Western Australia over the past fifteen years from Royal Perth and Fiona Stanley Hospitals. We obtained clinical information from patient records and as discussed at BMT meetings. The demographics and data regarding previous therapies, age, graft-versus-host disease and other medical issues was recorded.

Result

Fifteen patients have undergone allogeneic transplantation over the time period, all with Fludarabine and Melphalan conditioning (all having had BEAM autograft previously). Nine remain alive, eight in continuous remission. Two females have had successful pregnancies after implantation of frozen embryos, while a third female is pursuing a local surrogate carrying her frozzed stored embryo. . There were no pregnancy issues of concern, and hormonal supplementation was provided. Healthy babies were delivered of normal birth weight. One patient developed a flare of chronic graft-versus-host disease requiring increase in immunosuppression including steroids. The third patient who has received Brentuximab Vedotin and DLIs for relapse four years post allograft is pursuing the surrogate due to concerns about possible effects of pregnancy on disease progression with immune modulation.

Conclusion

Allogeneic transplantation for Hodgkin Lymphoma can provide successful long-term survival and enable fertility in cases where planning via storage of frozen embryos have occurred, in conjunction with fertility expert obstetricians. Regular reviews for pregnancy complications and graft versus host disease, particularly post-delivery, are required in this setting.

140. Palifermin, administered for 3 doses, minimises mucositis in stem cell transplantation patients conditioned with chemoradiotherapy.

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Background

Palifermin, a recombinant human keratinocyte growth factor with proven efficacy in mitigating mucositis in patients receiving chemoradiotherapy conditioning before haematopoietic stem cell transplantation (HSCT), is usually administered intravenously, 60mcg/kg/day, for 3 days before and after conditioning (total 6 doses).

Aim

To evaluate the effectiveness of palifermin administered for 3 doses only before chemoradiotherapy in patients undergoing HSCT as measured by the duration of parenteral nutrition (PN) and parenteral analgesia (PA) required in this group, compared with a similar cohort described in the published literature who received a total of 6 doses.¹

Methods

Patients receiving total body irradiation (TBI) and chemotherapy conditioning for HSCT within a single institution were eligible to receive 3 doses of palifermin. Medical records and dispensing histories were retrospectively reviewed over a two year period from June 2015 to May 2017. Effectiveness was assessed by measuring required durations of parenteral nutrition (PN) and parenteral analgesia (PA). Results were compared with a published retrospective study of 77 patients who received 6 doses of palifermin and TBI-based conditioning.¹

Results

Thirty-three patients with a median age of 39 years were included. The most common diagnosis was acute leukaemia in 29 patients. All patients received TBI, with 31 receiving a cyclophosphamide based regimen. Methotrexate, as graft versus host disease prophylaxis, was administered to 26 patients. PN was required by 25 patients (75%) and PA by 16 patients (48%). The mean (SD) duration of days on PN was 13 days (6.3) in the study group and 13 days (7) in the literature cohort (p=1.0). The mean (SD) duration of PA in the study group was 8.4 days (4.1) compared to 7 days (9) in the literature group (p=0.34).

Conclusions

Palifermin 60mcg/kg/day, intravenously administered for 3 days before chemoradiotherapy conditioning for HSCT, is effective in minimising mucositis as indicated by duration of PN and PA.

Reference:

- Goldberg, JD et al. Palifermin is efficacious in recipients of TBI-based but not chemotherapy-based allogeneic hematopoietic stem cell transplants. *Bone Marrow Transplantation* 2013; 48: 99-104

141. Adherence to institutional guidelines for total parenteral nutrition in patients undergoing autologous stem cell transplant

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Method

We performed a retrospective audit of 21 patients who received TPN while undergoing auto-HCT for lymphoma or myeloma between July 2015 and May 2017. Data were obtained from the patient medical records. The key criterion for the consideration of TPN was the achievement of $\geq 60\%$ of estimated nutritional requirements (EER%). Failure to achieve the target EER% for four days ("target EER% criterion") was considered an indication for TPN.

Results

21 patients received TPN between July 2015 and May 2017. A contraindication to enteral feeding was documented in all 21. The median time from stem cell infusion (SCI) to commencement of TPN was 8 days (range, 2-14). The median duration of TPN was 6 days (range, 2-15). 43% of patients received TPN for at least 7 days. EER% was documented on 67% of days prior to commencement of TPN. The EER% criterion for commencement of TPN was met in 66% of patients. In this group of patients, the mean time to commencement of TPN was significantly shorter (Table 1) and the mean duration of TPN was longer, with a trend toward statistical significance.

Table 1

EER% criterion met	Mean time from SCI till starting TPN (days)	<i>p</i>	Mean duration of TPN (days)	<i>p</i>
Yes	6.9	0.02	7.8	0.09
No	10.4		5.0	

Conclusion

We identified deficits in documentation of key criteria for commencing TPN (particularly EER%) that may result in delay in commencement of TPN and inadequate TPN duration. Only 43% of patients received TPN for more than the recommended duration. These deficits may lead to adverse nutritional outcomes as well as ineffective use of resources. Further work is required to ensure adequate documentation of key criteria.

142. Feasibility and sustainability of a cognitive rehabilitation program in survivorship care post-autologous stem cell transplantation

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Aim: Chemotherapy-associated cognitive impairment can adversely impact cancer survivors. Knowledge about its aetiology and therapy is limited. An online Cognitive Rehabilitation Program (CRP) has been reported to improve self-reported cognitive function in cancer survivors. This pilot study examines the feasibility and sustainability of a CRP in survivorship care post-autologous stem cell transplantation (ASCT).

Method: Eligible participants recruited for this multi-site study were administered a nine-week course of CRP, commencing day40±5 post-ASCT. Participants were evaluated using a neuropsychological tool (CogState) and validated questionnaires at baseline, pre-CRP (day40±5), post-CRP and six months post-CRP. Appropriate tests (e.g. Fisher exact test) were used for statistical analysis.

Results: Twenty-nine participants have been recruited thus far. At interim analysis, ten participants have completed the CRP (Table 1).

Table 1: Demographics (n=10)

Age	Sex	N	Previous chemo	N	%	Diagnosis	N	%
Mean (SD)	55.0 (12.4)		Yes	10	100	MM	6	60
Range	27-69		No	0	0	NHL	2	20
						HL	2	20

Participants reported high satisfaction and ease of use with the CRP (Table 2). Despite efficacy not being a primary endpoint, there was a trend demonstrating an increase in EORTC QLQ-C30 cognitive function scale post-CRP (Table 3), although no definite conclusion could be made in this pilot study.

Table 2: Mean score of self-reporting of CRP (n=10), maximum score 5

	6 Week Data (during CRP)	10 Week Data (post-CRP)
Easy to Learn to Use	4.31	4.56
Easy to Use	4.44	4.47
Satisfied	4.44	4.42

Table 3: EORTC QLQ-C30 Cognitive Function Scale, Mean (SD) scores (n=10)

	Baseline	Day40 (post-ASCT)	10 weeks (post-CRP)	Change
EORTC QLQ-C30 Cognitive Scale	78.3 (17.7)	71.7(19.3)	81.7 (16.6)	10

There were no changes in the mean scores for the stress and quality of life questionnaires. No participant reported significant anxiety or depression during the study.

Conclusion: This preliminary data suggests that the CRP might be a feasible tool to minimise the impact of chemotherapy-associated cognitive impairment post-ASCT and a future randomised study is under consideration.

143. Development & implementation of corrective action plan to address high micro-contamination rates for HPC(M)

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Introduction: Observed trend of contamination of HPC(M) collected via bone marrow harvest procedure at Fiona Stanley Hospital with 60% of HPC(M) products collected between Sep 2015 to Nov 2016 returning positive cultures. All contaminants were common skin commensals. Benchmarking data against similar collection facilities interstate/internationally currently not available.

Aim: Root-cause analysis, development and implementation of a corrective action plan including investigation of contamination source.

Methods:

- Multidisciplinary team consultation (BMT physicians, Infection Prevention Management team, Theatre Nurses, BMT Scientists)
- 1. Infection Prevention and Management (IPM) observation of Bone Marrow Harvest procedures to aid identification of potential causative actions contributing to microbial contamination. Assessment of the following practices throughout collection procedure:
 - Hand Hygiene Techniques & Standard Precautions
 - Aseptic Non-touch Technique (ANTT)
 - Preparation & maintenance of the sterile field
- 2. Microbial contamination investigation with cultures performed at intervals (eg. 1st draw, mid-collect) during the procedure to assess point of introduction of microbial contaminants.
- 3. Formation of a dedicated bone marrow harvest nursing team to assist with all harvest procedures
- 4. Implementation of additional precautions (Ioban™ antimicrobial dressings)
- 5. Review suitability of consumables
- 6. Refresher training & competency assessment of surgical scrubbing, aseptic gowning & double gloving
- 7. Microbial contamination data monitoring and trend analysis for HPC(M) Collection Procedure.

Results: HPC(M) Collections performed between September 2015 and November 2016 (n=10) had a contamination rate of 60% with the following organisms:

- S. epidermidis (n=2)
- Propionibacterium species (n=3)
- S. capitis (n=1)

Cultures performed at intervals identified contaminants were introduced mid-collect rather than from the 1st draw, reinforcing the importance of applying vigilance to maintain the sterile field throughout the procedure.

Early success observed after the implementation of corrective actions with consecutive negative results at our centre.

Conclusion: Multidisciplinary approach indicates early promising outcome of consecutive negative results since the implementation of corrective actions.

144. Autologous transplant using fresh HPC(A) as an alternative to manage clumping observed in cryopreserved HPC(A)

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Introduction: 37 year old male diagnosed with Multiple Myeloma in Feb 2016. Initial HPC(A) collected (June 2016) for planned autologous transplant. Collected cells were cryopreserved, with a clumping phenomenon observed during routine post-thaw viability assessment.

Aim: Investigate activity of the clumping phenomenon to verify safety of cryopreserved products for infusion.

Methods: Preliminary HPC(A) collection yield 5.7×10^6 /kg & cryopreserved. Post-thaw viability studies performed within 7 days of cryopreservation. Clumping not observed at point-of-thaw but developed as the product warmed to room temperature. Clumping suspected to be fibrin associated-following investigations performed:
-Repeated post-thaw viability (retention vials & cryopreserved bags)
-Filtration
Although viability & CFU-GM within acceptable limits, products deemed unsuitable for use.

Secondary HPC(A) collection yield = 8.7×10^6 /kg stored overnight at 4°C and cryopreserved the following day. Clumping not observed post-overnight storage or during processing. Clumping phenomenon again observed during post-thaw viability studies. Further investigations:
-Post thaw-washing procedures at 4°C on cryopreserved products
-Platelet function, coagulation studies & cryoglobulin testing. Second collection unsuitable for infusion.

Third autologous HPC(A) collection scheduled, followed by immediate myeloablation & infusion of fresh HPC(A) (<48 hours of collection, stored at 4°C). Donor family search performed – sister identified (HLA match) in the event of the clumping phenomenon occurring with refrigerated cells.

Results: Clumping was observed in all post-thaw investigations. Clumping could not be prevented by filtration nor post-thaw washing. No abnormality detected for Cryoglobulins, Platelet function & coagulation studies. Third autologous collection of HPC(A) = 4.4×10^6 /kg. Clumping not observed in fresh HPC(A) stored at 4°C and infused <48 hours of collection. Successful neutrophil engraftment ($>0.5 \times 10^9$ /L) achieved on Day 10 post-transplant.

Conclusion: While the aetiology of the clumping wasn't identified, observing the thermal activity of the clumping phenomenon assisted with achieving a positive outcome. This case demonstrates the use of fresh autologous HPC(A) as an effective alternative to cryopreserved products for transplantation.

145. Allogeneic transplantation for ALL in Western Australia – favourable outcomes in CR1

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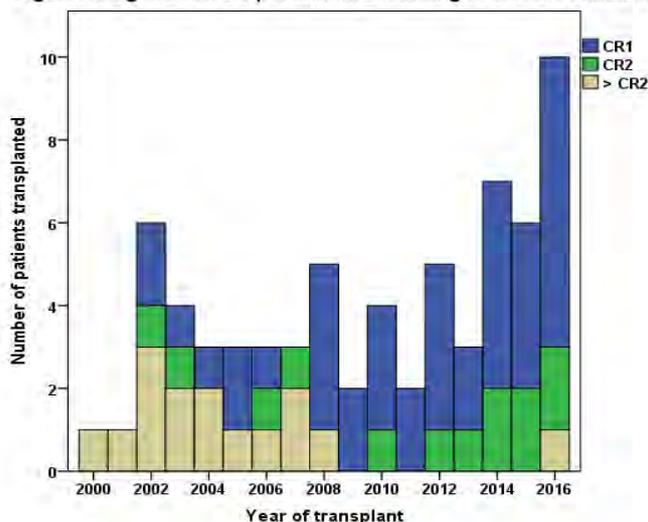
Aim: To review trends and outcomes of allogeneic haemopoietic progenitor cell transplantation (HPCT) for acute lymphoblastic leukaemia (ALL) at a state adult transplant service.

Methods: Sequential patients who received allogeneic HPCT for ALL between 2000-2016 were identified from a prospectively maintained transplant database.

Results: 71 patients underwent HPCT for ALL between 2000 - 2016. The volume of HPCT increased over time for patients in CR1 (first complete remission), while the number performed for more advanced disease was steady (CR2) or declined (>CR2) (Figure). Median age at transplant was 35 years (range 19-61), and did not change over time. Median follow-up was 47 months, and 4-year overall survival was 48%. Survival was higher in patients transplanted in CR1 (CR1 66% vs. CR2 31% and 13% beyond CR2, $p < 0.001$). Patients were significantly more likely to relapse if transplanted after CR1 (CR1 14% vs CR2 31% and 54% beyond CR2, $p = 0.012$), while non-relapse mortality was not associated with disease stage ($p = 0.28$). In multivariate analysis, age, cytogenetics and year of transplant did not significantly impact overall survival.

Discussion/Conclusion: Despite recent interest in the role of chemotherapy-only regimens for ALL, we have observed a progressive rise in the number of HPCT performed in CR1. Survival outcomes observed in this group compare favourably to other treatment modalities, including paediatric-style intensive chemotherapy regimens, and appear to justify early HPCT. However, patients who were transplanted in CR2 or beyond had much higher risk of relapse and inferior survival. While the availability of novel salvage agents such as blinatumomab may facilitate HPCT in CR2 or beyond, it remains uncertain if outcomes in this group of patients can be significantly improved. Moving forward, the ability to detect minimal residual disease will assist in risk stratifying and identifying patients who will benefit from HPCT for ALL.

Figure: Allogeneic HPCT per annum according to disease status at transplant



146. Severe Acute GVHD following Allogeneic Stem Cell Transplant post Nivolumab therapy

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Aim

To describe a case of severe acute graft versus host disease (aGVHD) after allogeneic stem cell transplant in a patient with HL previously treated with Nivolumab (anti-PD1 antibody).

Case report

A 49-year-old male underwent a reduced intensity allogeneic stem cell transplant with Flu/Mel/TBI from his HLA matched brother for relapsed refractory classical nodular sclerosing Hodgkin Lymphoma. Prior lines of therapy included ABVD, IGEV, BEAM Auto HSCT, Nivolumab and Brentuximab. 6 cycles of Nivolumab were administered from July to September 2016 prior to Allogeneic HSCT (November 2016). Following an uneventful initial post transplant course, he was admitted on day +25 with profuse diarrhoea, vomiting and acute kidney injury. Colonoscopy and biopsy confirmed grade 4 gastrointestinal acute GVHD. Initial therapy with methylprednisolone and tacrolimus was ineffective and IV mycophenolate and SC entanercept were introduced on day +5. Symptomatic therapy included octreotide infusion, loperamide and codeine. His disease remained unresponsive and his course was further complicated by peptic ulceration and GIH, microangiopathic haemolytic anaemia, hepatic failure and pancytopenia. Despite maximum treatment, including administration of rituximab on day +61 and alemtuzumab on day +68 he continued to deteriorate, and passed away on day +70.

Discussion

There is limited literature describing the outcomes and management of patients previously exposed to immunotherapy undergoing allogeneic HPT and particularly the incidence, prophylaxis and treatment of aGVHD in this setting. Small retrospective series suggest that allogeneic HPT after PD-1 inhibitor therapy is feasible, does not negatively affect engraftment, and provides acceptable relapse rates but that patients may be at increased risk of early immune toxicity (including aGVHD) and Hepatic SOS (Merryman et al).

Conclusion

Allogeneic HPT post PD-1 blockade is feasible but appears to confer greater toxicity and transplant-related mortality. Further research is required to the optimal prophylaxis and treatment in patients exposed to checkpoint inhibitors pre-HPT.

147. Blood stream infections following allogeneic BMT: A single centre experience

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Aim

We examined the rates of confirmed blood stream infection (BSI) in allogeneic BMT patients, the organisms isolated, and the association of BSI with other post-transplant outcomes.

Method

184 patients in an observational cohort of allogeneic BMT recipients treated at the RBWH between 2009 and 2015 were studied. We have examined all blood cultures (BC), circumstances of collection, BSI rate, organisms, antimicrobial resistance, other infections and transplant outcome. Empiric antimicrobials were piperacillin-tazobactam ± vancomycin for the majority, with adjustments for prior colonisation or infection. Contingency analyses were used to assess statistical significance.

Results

Median patient age was 52 ± 14 years (58.7% male), with 35.3% of transplants for AML, 18.5% for ALL and 16.8% for MDS. 167 (90.8%) patients had blood cultures (BC) drawn between *d*-7 and *d*100. There were 777 unique episodes of BC collection and 217 (27.9%) yielded positive results, 111 (14.2%) for clinically significant organisms. Gram-negative organisms were isolated in 58.1% of cases, with 38.8% resistant to piperacillin-tazobactam (via intrinsic and acquired mechanisms). Extended spectrum beta-lactamases were present in 7.4%, and meropenem resistance in 11.1%. Piperacillin-tazobactam resistance was observed in 40% of the 35 Gram-positive isolates, attributable to VRE (4 episodes), MRSA (1 episode), *Staphylococcus epidermidis* (4 episodes) and the remainder due to viridans streptococci (5 episodes). Anaerobes and fungi represented 4 cases (4.3%).

The majority of BC were collected in febrile patients (62% of episodes; T≥38°C) and these yielded significant organisms more frequently than afebrile episodes (16.8% vs 9.3%; OR 1.9, 95% CI 1.21 – 3.23; p=0.0058). Neutropenia, timing of episode (relative to engraftment), TPN, hypotension and CMV reactivation were not associated with BSI. Higher grade III-IV aGVHD rates were observed among patients with confirmed bacteraemia (OR 2.79, 95% CI 1.18 – 6.05; p = 0.0213).

Conclusion

We report low rates of BSI compared with published literature, but higher than expected rates of resistance to empiric antibiotics in both Gram-positive and Gram-negative isolates. These data provide guidance for management of febrile episodes after BMT.

149. Autologous haematopoietic stem cell transplantation (aHSCT) in multiple sclerosis: a phase II trial.

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Aim

To evaluate the safety and efficacy of autologous hematopoietic stem cell transplantation with high-intensity immunosuppression in patients with multiple sclerosis (MS).

Methods

A Phase II clinical trial of aHSCT using a BEAM + ATG chemotherapeutic regimen for patients with relapsing remitting and secondary progressive MS commenced at St Vincent's Hospital in December 2010. Patients eligible for aHSCT had an Expanded Disability Status Scale [EDSS] of 2.0–7, had failed two prior disease-modifying therapies and displayed evidence of ongoing disease activity manifesting as clinical relapses and/or new MRI lesions in the year prior to aHSCT.

Results

Twenty-four patients with relapsing remitting MS and 12 with progressive MS completed aHSCT to date with a median follow-up of 23.77 months (range 2.73-63.16). 55.6% of patients had received ≥ 4 disease modifying therapies prior to aHSCT with 63.8% having received Natalizumab. Progression-free survival, clinical relapse-free survival, and MRI activity-free survival for the entire cohort are 77.8%, 91.7% and 91.7% respectively. Progression free survival in the RRMS cohort is 91.7%. Treatment related mortality was 0%. Adverse effects due to aHSCT were consistent with expected toxicities. Two patients required admission to intensive care units in the post-transplant period. There were no significant late neurologic adverse effects noted. Changes were noted in neurologic disability with 14/36 (38.9%) of patients demonstrating a sustained (> 6 months) improvement in EDSS [1]. 6 SPMS and 2 RRMS had worsening of disability as measured by EDSS.

Conclusion

aHSCT was effective for inducing long-term sustained remission in the majority of active RRMS and SPMS patients within our heavily pre-treated cohort of patients. Age <35 yrs and baseline EDSS score <4 had the highest rates of EFS. The results support revision of criteria to allow identification of those patients that will derive the greatest benefit from aHSCT.

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150. Activity and capacity profile of transplant physicians and centres in Australia and New Zealand

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Aim

To analyse and report on indicators of haematopoietic cell transplant (HCT), physician time use and HCT centre activity.

Method

HCT centres in Australia and New Zealand (A&NZ) were invited to provide demographic and time use details for physicians participating in HCT patient care (HCT physicians). Resource details for all centres were also collected.

Results

From a total of 46 centres that were invited to participate, completed data were received from 37 (80%) representing 185 HCT physicians, with a median age of 48 (range 33-72), of whom 31% were female. Just over half of HCT physicians cited prior work experience in large overseas HCT centres (97, 52%) and over one-third (79, 43%) possessed postgraduate qualifications other than specialist training. Total annual average HCT per HCT physician FTE was 14.2 for centres performing both allogeneic and autologous HCT, 6.6 for autologous-only centres and 10.6 for all centres. For all HCT physicians surveyed, the average proportion of time spent on HCT related tasks was 31.7%, while for HCT physicians at allogeneic + autologous centres this was 43.9% and for those at autologous-only centres this figure was 18.9%. For centres that perform both allografts and autografts, there were averages of 4.0 allogeneic HCT annually per HCT bed, and 7.1 allogeneic HCT annually per HCT physician FTE. Projections of the A&NZ HCT physician workforce indicated that the numbers of HCT physicians are likely to stay within the region of 170 to 190 for the next 10 years, while HCT activity will likely continue to climb steadily.

Conclusion

Healthcare and government authorities should be prepared to enable and support HCT activity of greater numbers and complexity in A&NZ in the future.

151. Haematopoietic stem cell transplantation-associated thrombotic microangiopathy: A deadly complication requiring early diagnosis and management

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Aim: To report on four patients at a single centre who developed post haematopoietic stem cell transplantation (HSCT) thrombotic microangiopathy (TA-TMA) and were treated with Eculizumab; ¹ a monoclonal antibody directed towards C5 that inhibits the formation of the C5b-9 membrane attack complex.

Method: Retrospective analysis of allogeneic HSCT recipients who developed TA-TMA and commenced on Eculizumab at our unit over a twelve month period. Diagnosis of TA-TMA involved detection of red cell fragmentation, de novo or prolonged thrombocytopenia and an elevated LDH. Renal dysfunction including proteinuria and gastrointestinal manifestations were included in the assessment as were risk factors such as acute graft-versus-host disease (aGVHD) and the use of calcineurin inhibitors.² Atypical haemolytic uraemic syndrome (aHUS) genetic testing was performed on two of our patients.

Result: Four patients were diagnosed with TA-TMA a median of 6 weeks (range 5-17weeks) after transplant and had cyclosporin therapy withdrawn. Unfortunately non-relapse mortality was significant in this cohort (50%). Two patients received plasmapheresis prior to successful Eculizumab therapy. Despite the lack of access to real time complement testing and Eculizumab levels, we safely utilised CH50 suppression during maintenance Eculizumab dosing to guide cessation of treatment without recurrence of the TMA in two of our treated patients. Two patients had aHUS genetic testing; one patient did not have any detectable pathogenic variant in their genetic panel whilst the other had a heterozygous deletion encompassing exons 3 to 6 of the CFHR1 gene.

Conclusion: Our cohort of patients demonstrates that TA-TMA is a serious and life-threatening complication of HSCT. The diagnosis and management of these patients is challenging, especially in the setting of concurrent aGVHD. Early diagnosis requires a high index of suspicion and close monitoring after HSCT. Eculizumab may be successfully instigated in patients with severe TA-TMA.

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152. Does perceived control impact patient experience of distress and coping in bone marrow transplantation?

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Aim: Stress, depression and anxiety negatively impact recovery from allogeneic bone marrow transplant (BMT) (Foster et al, 2009). Perceived control has been shown to mediate the impact of traumatic events on levels of distress (Frazier et al, 2011) and affect an individual's capacity for adaptive coping in cancer (Kvillemo & Branstrom, 2014). Allogeneic BMT is as stressful event inherently low in personal control. Improving a person's perception of control may improve psychological functioning and contribute to more adaptive coping strategies. This study aims to determine if perceived control has a similar impact on the experience of psychological distress and coping in BMT.

Methods: 100 participants, aged between 18 and 70 years, will be recruited within 3 years of allogeneic bone marrow transplant. Participant will complete a series of self report measures including the Depression, Anxiety and Stress Scale (DASS-21; Lovibond & Lovibond, 1995); Perceived Control Over Stressful Life Events Scale (Frazier et al, 2011); COPE (Carver et al, 1989); and the Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) scale (McQuellon et al, 1997). Simultaneous regression analyses will be used to assess the relationship between the subscales of perceived control, distress and coping.

Results: Results were unavailable at time of abstract submission.

Conclusion: Should results be consistent with previous research, therapeutic programs aimed at improving perceptions of perceived control could be designed to reduced distress and enhance coping resources during and post transplantation.

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153. Laboratory evidence of immune disease post allogeneic stem cell transplant (HPT).

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Aim

The development of auto immune diseases post allogeneic stem cell transplant is well described. However the clinical utility of screening for immunological disorders post-HPT is uncertain. We report the results of immunological screening tests performed post-HPT and the association of these with development of clinical features of autoimmune disease (AD).

Method

We retrospectively reviewed the results of antinuclear antibody (ANA), rheumatoid factor (RF), double stranded DNA, extractable nuclear antigen antibodies (ENA), anti-mitochondrial antibodies (AMA), thyroid autoantibodies (anti-TPO), liver kidney microsomal antibodies (anti LKM) and anti-thyroglobulin antibodies in 100 recipients of allo-HPT who had survived at least 1 year post-transplant.

Results

Preliminary analysis of 100 survivors of allo-HPT found that 13 were positive for anti-TPO and 9 for anti-thyroglobulin antibodies while 17 patients had evidence of a positive ANA, 4 a positive ENA, 1 a positive AMA, and 2 high titre RF. 4 patients were ANA positive (without clinical significance) pre transplant, and negative post-transplant. 1 patient had seronegative rheumatoid disease pre-HPT and 2 patients had a multinodular goitre with anti-TPO antibodies pre-HPT (both of whom still had anti-TPO post-HPT). No patients developed new AD post HPT.

Conclusion

Serological evidence of AD occurs commonly post-HPT but these are rarely associated with clinical AD. While longer follow-up with larger patient numbers is required to more fully explore the clinical relevance of immunological testing post-HPT, these results suggest that laboratory evidence of immunological disease post-HPT may be epiphenomena associated with immune dysregulation post-HPT and may be of little clinical relevance. This raises questions about the clinical utility of screening for AD post-HPT.

154. High mucositis risk bone marrow transplant conditioning regimens are associated with lower serum cyclosporin levels

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Aims

Serum cyclosporin levels have been shown to influence allogeneic haematopoietic stem cell transplant (allo-HSCT) outcomes including graft rejection, graft-versus-host disease, and disease relapse. The risk and extent of cyclosporin malabsorption following mucositis has not been assessed. This study aims to assess the incidence of severe (grade 3 and 4) mucositis following various conditioning regimens and the effect of post-transplant mucositis on serum cyclosporin levels.

Methods

169 patients received myeloablative and reduced-intensity allo-HSCT at the Royal Melbourne Hospital between 2001 and 2013. The proportion requiring total parental nutrition (TPN) and mean TPN duration were used as surrogate markers for the incidence and severity of grade 3 and 4 mucositis. Serum cyclosporin levels (2 hours post administration) following transition to oral administration in the first three months following transplant were compared across conditioning regimens using the Mann-Whitney U test.

Results

The proportion of patients and mean duration of TPN largely correlated with increased intensity of conditioning regimen. In increasing order of intensity: (conditioning regimen, TPN patients[proportion], mean TPN days) FluMel, 17/31[49%], 11.6 days; BuCy, 45/56[76%], 11.6 days; CyTBI, 8/12[67%], 13.0 days; EtoTBI, 64/71[89%], 20.4 days. Median cyclosporin levels negatively correlated with increasing intensity of conditioning regimen: FluMel (1039 ng/mL), BuCy (1009 ng/mL), CyTBI (793 ng/mL), EtoTBI (507.7 ng/mL). Comparisons to FluMel as the least mucositic regimen were as follows: BuCy, $p=0.753$; CyTBI, $p=0.120$; EtoTBI, $p<0.0001$. Linear regression analysis showed an inverse correlation of TPN duration with median cyclosporin levels ($R^2=0.23$, $p<0.0001$).

Conclusion

In our patient cohort, mucositis risk increased with conditioning regimen intensity. Increased incidence and duration of TPN use was associated with lower median serum cyclosporin levels in the first three months following transplant. These variations in cyclosporin levels have implications for the interpretation of transplantation outcomes.

155. Effect of Nucleated Cell Concentration on Peripheral Blood Stem Cell Yield and Viability

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Aim

Previous studies have reported that high number of nucleated cell counts in cryobags can reduce the viability of CD34 positive cells in relation to time passed prior to cryopreservation. This observation has not been consistent as a few other studies have shown that high cell concentrations are well tolerated without compromising the post thaw viability of stem cells and clinical outcomes. The primary objective of this study is to evaluate the effect of nucleated cell concentration on CD34 positive cells yield and viability prior to cryopreservation. The secondary objective is to evaluate the effect of nucleated cell concentration on clinical outcomes as defined by the time to platelet and neutrophil engraftment.

Method

Retrospective laboratory data including nucleated cell concentration, CD34 positive cells yield and post-thaw viability were collected at two autologous transplant centres in South East Queensland, one which implemented routine measures to control the harvested nucleated cell count to $\leq 300 \times 10^6/\text{ml}$ and one which did not implement such measures. Statistical analyses were performed to evaluate the correlation between nucleated cell concentration and CD34 positive cells yield and post-thaw viability. These data were compared across the two centres. Correlation studies were also performed on nucleate cell concentration and clinical outcomes including the time to platelet and neutrophil engraftment.

Result

Preliminary result showed no significant correlation between nucleated cell concentration and CD34 positive cells yield and post-thaw stem cell viability. Nucleated cell concentrations also did not predict the time to platelet and neutrophil engraftment.

Conclusion

Some laboratory implement measures to limit the harvested nucleated cell concentration to prevent compromising the viability of harvested stem cells. In our study, we have found no significant correlation between these variables. Larger studies will be needed to confirm our findings as this may impact on routine laboratory practice and recommendations in quality improvement programme.

156. Comparison of Dinakara and Cotes method on correction of diffusion limit of carbon monoxide and the impact on Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI)

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Aim

Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) described by Sorror 2005 provides risk stratification for transplant recipients. Respiratory comorbidity, which takes into account diffusion limit of carbon monoxide (DLCO) and forced expiratory volume in 1 second (FEV1), is the most common comorbidity scored on the HCT-CI. The original publication utilised Dinakara method for correction of DLCO to haemoglobin concentration (Hb). Recommendation of the American Thoracic Society (ATS) and European Respiratory Society (ERS) and therefore, our institution utilises the Cotes method for correction. We compared the effect of Dinakara and Cotes method on correction of DLCO, HCT-CI scoring and non-relapse mortality (NRM).

Method

We retrospectively collected HCT-CI scores, relapse, deaths from 195 consecutive allogeneic stem cell transplants at the Royal Brisbane and Women's Hospital between May 2014 and May 2016 with median follow up of 394 Days. Non-relapse mortality defined as any death in the absence of relapsed disease.

Results

During our follow up period, 44 patients relapsed and 50 deaths occurred with median survival not reached. When utilising Dinakara method for scoring respiratory co-morbidity, patients who scored 2(moderate) or 3(severe) was 22.1% and 8.7% respective. However, by utilising the Cotes method, this increased the moderate and severe respiratory co-morbidity to 43.1% and 23.1% respectively. By utilising the Cotes instead of Dinakara method, number of patients classified as high risk HCT-CI increased from 32.3% to 49.7%.

There were no significant difference in non-relapse mortality between the two methods at Day 100 or 2 years. The high risk patients had NRM using Dinakara vs Cotes of 9.2 % vs 10.1% at day 100 and 16.9% vs 22.8% at 2 years.

Conclusion

Utilising cotes method rather than Dinakara method for the correction of DLCO significantly increased the number of high risk patient on HCT-CI, however, there was no significant impact on non-relapse mortality.

157. Effect of over-night storage, plasma reduction and nucleated cell number on viable CD34+ cell recovery

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Aim

To study the effect of plasma reduction and over-night storage on the relationship of total nucleated cell counts (NCC) and viable CD34⁺ (vCD34⁺) recovery in haemopoietic progenitor cell (HPC) harvests.

Method

Data from 375 HPC apheresis harvests, collected in the Haematology department at the Calvary Mater Newcastle Hospital, were assessed to see if total NCC at collection and after plasma reduction, in combination with over-night storage at 2-8°C, influenced the recovery of vCD34⁺ after cryopreservation. Univariate and multivariate analysis were performed to assess for differences between groups.

Results

NCC of >300x10⁶/mL in cryopreserved harvests negatively impacts on vCD34⁺ recovery, but this was not statistically significant and is highly variable ($P=0.21$). This result was assumed to be influenced by a factor other than NCC. Multivariate analysis of NCC at harvest and after plasma reduction +/- over-night storage, and vCD34⁺ cell recovery found those harvests with >300x10⁶/mL NCC after plasma reduction in combination with over-night storage resulted in a loss of vCD34⁺ cells which was statistically significant ($P=0.02$).

Conclusion

Current protocols for NCC in cryopreserved samples are <400x10⁶/mL due to the lower recovery of erythroid burst forming units¹ when NCC are at a higher concentration. If stored over-night at 2-8°C, it is suggested that autologous HPC harvests be diluted with autologous plasma or Albumex 4 if NCC are >300x10⁶/mL². Preliminary analyses of our data support these protocols, but only when over-night storage is a factor.

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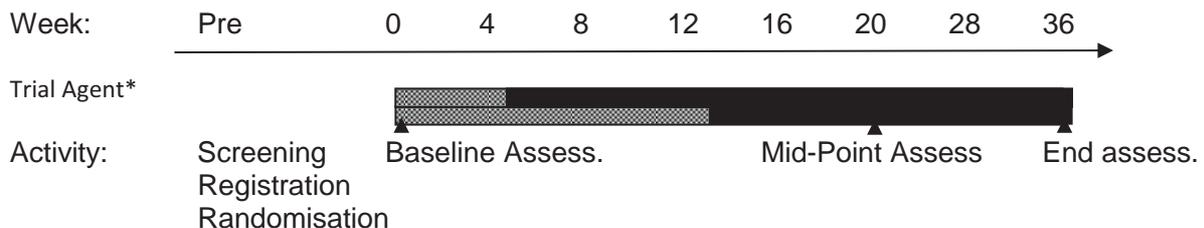
158. A pilot study of green tea polyphenols in untreated, early stage chronic lymphocytic leukaemia

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Aims: To assess the effect of green tea polyphenols (GTP) in patients with early stage CLL with regards (1) Clinical efficacy, (2) Toxicity and (3) Quality of life (QOL).

Methods: Phase IIA, single-arm, 36 week, staggered, crossover trial of oral green tea (GT) extract versus placebo in patients with early stage CLL who do not require treatment as per NCIWG recommendations. Placebo-controlled run-in period was 4 weeks in cohort A and 12 weeks in cohort B (see figure). Two capsules of compounded GT extract or placebo were administered with morning and evening meals (1400mg/day total; equivalent to 9 cups of green tea). Primary end-points included (1) Clinical efficacy as assessed by (i) reduction in absolute lymphocyte count (ALC), and (ii) reduction in palpable lymphadenopathy as assessed by SPD, (2) Toxicity and, (3) QOL as assessed by EORTC-QLC 30.



*Shaded = placebo, black = green tea extract.

Results: Twenty one patients were enrolled on the trial. Eighteen patients completed the course of oral GT extract as prescribed. The majority of patients were male (78%) with Binet stage A (83%). No patients achieved a pre-defined biologic response as measured by a sustained $\geq 20\%$ decline in ALC and/or a $\geq 30\%$ reduction in the sum of the products of all palpable nodes. There was also no significant change in CD19/CD5+ lymphocytes from baseline to end assessment. Two patients withdrew from the study (Sneddon-Wilkinson skin rash and mouth ulceration). Grade I transaminitis occurred in 5 (27%) patients. QOL data is being collated and will be updated at time of presentation.

Conclusion: Administration of daily oral green tea extract at a dose of 1400mg/day did not result in clinically significant reductions in absolute lymphocyte count, CD19/CD5+ lymphocyte count or palpable lymphadenopathy in patients with early stage CLL.

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159. Neurological toxicity in combination btk/PD-1 inhibitor therapy

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Therapy with immune checkpoint inhibitors is increasingly used in malignancy, leading to the emergence of a different range of toxicities compared to that observed with conventional chemotherapy agents. Nivolumab and other PD-1 inhibitors have been associated with autoimmune sequelae encephalitis

We describe the case of a 65 year old man with chronic lymphocytic leukaemia (CLL), previously treated with fludarabine, cyclophosphamide and rituximab in 2013. In 2017, due to relapsed disease he commenced a clinical trial involving concomitant use of novel Bruton's tyrosine kinase (btk) and PD-1 inhibitors (BGB-3111/A317 trial).

Approximately six weeks after commencing treatment, the patient developed confusion and loss of attention, then with progressive neurological deterioration including muscle twitching, culminating in coma. Following investigations including brain imaging, meningeal biopsy, neuronal antibodies, electroencephalography and lumbar puncture, the working diagnosis was of autoimmune encephalitis likely secondary to one or more of the therapeutic agents. Infection was excluded. Although CSF analysis did reveal the presence of a small CLL clone, the additional investigations as above were not in keeping with CNS malignancy as the cause for the presentation.

Despite neurologic response to pulsed methylprednisolone, he relapsed with decreased conscious state on attempted weaning to 100mg oral prednisolone. Therefore, additional steroid-sparing measures were instituted including intravenous immunoglobulin, high dose cyclophosphamide, and intrathecal hydrocortisone/methotrexate. Oral steroids were weaned gradually over five weeks, and oral cyclosporine was commenced. To date, he has made a near complete neurological recovery and has not been re-challenged with the agents.

This case highlights the potential for autoimmune toxicity with immune checkpoint inhibitors. Here, a life-threatening situation was successfully managed using several therapies not previously described in the treatment of severe neurological adverse events related to PD-1 blockade. We postulate that the patient's presentation is a consequence of a disinhibited immune response to either infection or CLL within the CNS.

160 Ibrutinib in the management of relapsed/refractory chronic lymphocytic leukaemia (CLL): a retrospective audit

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The management of CLL has experienced a paradigm shift with the development of targeted therapies. Ibrutinib, a Bruton tyrosine kinase inhibitor, was one of the first to be used in clinical practice. Its role in the management of elderly and comorbid patients who have relapsed/refractory CLL has been shown to be more effective than the conventional agent, chlorambucil [1]. Our aim was to establish our own outcomes of using ibrutinib in CLL and compare these with the literature [2].

30 CLL patients were identified on ibrutinib in our DHB. 27 patients had data available. Data was collected retrospectively on demographics, baseline and follow-up complete blood count (CBC) parameters and outcomes. GraphPad Prism 7.03 was used for Kaplan-Meier analysis.

63% of our patients were male and a significant proportion had advanced disease at diagnosis (44%) with high risk genetic markers (26%) (Figure 1). Median treatment duration was 7 months (range 1-23 months). More than half of our patients had received ≥ 2 treatments (Figure 2).

Male	63 % (17)
Age (median, years)	73 (range 49 – 90, years)
Stage at diagnosis (Binet)	
A	19 % (5)
B/C	44 % (12)
not known	37 % (10)
Genetic abnormalities	
Poor risk (TP53, del17p, del11q)	26 % (7)
Standard/good risk (trisomy 12, del13q)	44 % (12)
Genetic risk not known	30 % (8)
≥ 2 comorbidities (IHD, CKD, T2DM)	67% (18)

Figure 1 – Patient demographics

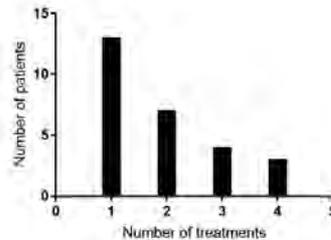


Figure 2 – Number of previous treatments received.

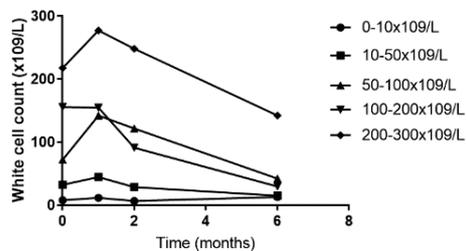


Figure 3 – White cell count response with Ibrutinib

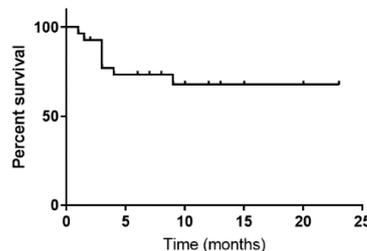


Figure 4 – Overall survival on ibrutinib. Median survival not reached

70% of patients remained on ibrutinib, showing a good response in CBC parameters (Figure 3); 30% (8/27) stopped ibrutinib due to toxicity or disease progression. 8 patients died; 7 of whom had discontinued ibrutinib (Figure 4). Median survival was not reached during our treatment period.

This audit showed that our mortality outcomes, although over a shorter time period, were similar to those in the literature for patients with relapsed/refractory disease on ibrutinib [2]. Of note, while the majority of our patients remained on ibrutinib with a good clinical response, those who discontinued had a poor outcome, reflected in studies on ibrutinib discontinuation [3]. Our aim now is to continue to monitor our patients on ibrutinib over a longer period of time. We await with interest the introduction into clinical practice of newer targeted therapies for CLL.

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161. Chronic lymphocytic leukaemia cytogenetics in Western Australia 2011-2016

Giguere-simmonds P¹, De Kraa R^{1,2}, Wright M¹

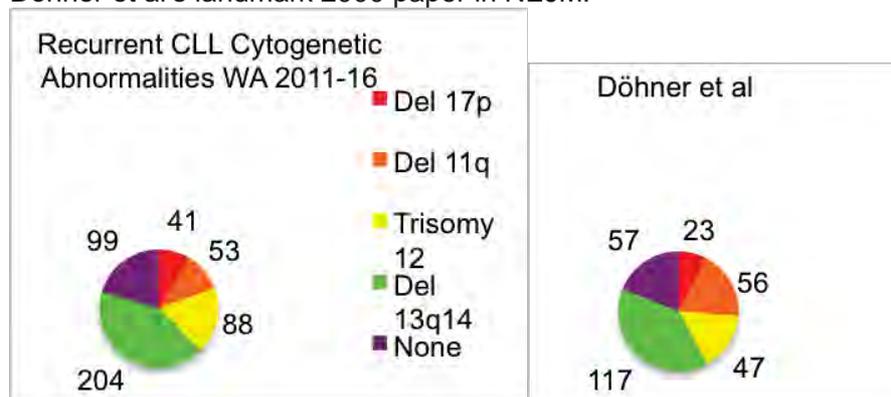
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Background: In patients with CLL requiring therapy, FISH tests for well-described recurrent cytogenetic abnormalities are recommended as a key prognostic indicator. The presence of specific recurrent cytogenetic abnormalities can also affect key treatment decisions including consideration of future allogeneic haematopoietic stem cell transplantation and novel agent therapy.

Aim: We aimed to determine the frequency of tests, and the incidence of the recurrent cytogenetic abnormalities in Western Australian patients with CLL for comparison with previous studies and to guide further study in CLL cytogenetics in a Western Australian (WA) context.

Method: Almost all cytogenetic tests in WA are performed by the Pathwest cytogenetics laboratory, now situated in FSH. Its database was examined over the period of 2011-2016 inclusive. All assays performed on CLL patients that included FISH probes for del17p, del11q, trisomy 12, del13q14 were counted and the number of patients positive for each abnormality were represented as a proportion of the total.

Results: 496 FISH panels were performed on WA CLL patients assaying for the presence of the recurrent cytogenetic abnormalities. There was an increase in testing in 2015-16 compared with 2011-2012. 11 patients had repeat testing. Of the 485 non-repeated panels, 41 (8.5%) demonstrated Del17p, 53 (10.9%) demonstrated Del11q (without 17p), 88 (18.1%) demonstrated Trisomy 12 (without Del17p, 11q), 204 (42.1%) demonstrated Sole 13q14 deletion, 99 (20.4%) possessed no recurrent abnormality. We compare these to the results of Dohner et al's landmark 2000 paper in NEJM.



Conclusion: Cytogenetic testing for CLL in WA has been relatively infrequent and recurrent testing at relapse is typically not performed. Besides the Del11q group, there is similarity to Dohner et al's original cohort of German CLL patients in terms of the proportions of clones bearing the recurrent cytogenetic abnormalities, suggesting these remain relatively preserved between populations. These results may guide further study of Australian CLL cohorts and provide insight into possible demands on treatment faced by health providers in an Australian setting.

162. Trisomy 12 in chronic lymphocytic leukaemia: characterisation of a local patient cohort

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Background: Trisomy 12 (+12) is a recurrent genomic aberration in chronic lymphocytic leukaemia (CLL) and confers an intermediate prognosis¹. Trisomy 12 CLL cases are unique, with increased expression of the homing integrin CD49d2-4 and strong association with NOTCH1 mutations⁵⁻⁷. Although +12 is identified recurrently in CLL, has prognostic significance and has unique genetic and phenotypic associations, its contribution to CLL leukaemogenesis and pathogenesis are unknown.

Aim: To characterise the clinical, genetic and immunophenotypic profile of a local cohort of CLL patients with +12.

Method: Next-generation sequencing (NGS) using a targeted 5-gene panel (NOTCH1, TP53, SF3B1, ATM, BIRC3) was performed on cryopreserved peripheral blood mononuclear samples from +12 CLL patients (Illumina MiSeq). The immunoglobulin heavy chain gene variable region (IGHV) mutational status was determined using the Lymphotrack assay. Flow cytometry using a specialised integrin panel (including CD49d) was also performed. Diagnostic SNP microarray data and cases notes were interrogated.

Results: 21 patients with +12 CLL and available samples have been identified. 81% are male with a median age of 67. 84% have an unmutated IGHV and 55% have advanced Rai stage. Trisomy 12 is present as a high frequency subclone (>60% of cells) in 74% of cases and is associated with an additional cytogenetic abnormality in two-thirds of cases. 17% of patients have an associated deletion of 14q. One of 10 patients in the training cohort harbours the classic NOTCH1 mutation (c.7541_7542delCT). NGS data analysis is ongoing. No difference in mean fluorescence intensity of CD49d was detected between 6 +12 and 5 normal karyotype CLL comparators (p=0.1125). CD49d expression was bimodal in 2 +12 cases.

Conclusion: Trisomy 12 is a marker of a unique subtype of CLL with unknown pathogenesis. The in-depth analysis of the local patient cohort is ongoing. Future aims include expansion of numbers and functional analysis of NOTCH1 mutants.

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163. A single centre retrospective clinical audit of the side effect profile of ibrutinib.

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Background/ Aim

The Bruton's tyrosine kinase (BTK) inhibitor ibrutinib has shown promising results in relapsed or refractory chronic lymphocytic leukaemia (CLL) and Mantle Cell Lymphoma (MCL) patients. We evaluated the 'real-life' side effects profile experienced by 20 patients taking ibrutinib for CLL or MCL via a medications access program (MAP) at the Fiona Stanley Hospital in Perth with the view to determining if they were similar in incidence and grade to those reported in phase 3 trials.

Methods

In this single centre study, we completed a retrospective clinical audit of all patients enrolled in the MAP. Data was collected from the pharmacy dispensation system, the clinical results interface and the digital medical record with sources including outpatient clinic letters, phone calls to clinic nurses, inpatient admission records including medications charts and pharmacist reconciliation forms. All reported side effects were recorded and were possible graded according to level of severity. Concurrent medications that were likely to contribute to side effects or interact with ibrutinib were also recorded.

Results

A total of 20 patients have been prescribed ibrutinib at FSH to date. With these 20 patients, there were 14 grade 3 / 4 reactions including infection, bleeding, neutropaenia and a seizure. Of these reactions, 9 resulted in admission to hospital and thus far there have been no fatalities with one admission still ongoing at time of writing. Further analysis is pending however interestingly there were no new atrial fibrillation reported and only one case of gastrointestinal disturbance; the latter likely due to lack of reporting as this was a common side effect in trials.

Conclusion

This audit demonstrates that similar to the registration trials, ibrutinib is a highly effective drug for CLL and MCL. Like all kinase inhibitor therapies, patient characteristics and co-morbidities need to be considered prior to commencing therapy. Careful consideration of concurrent medication is also required due to the unique side effect profile of ibrutinib and the potential for drug interactions.

165. The role of SMG1 and ATM in B cell lymphoproliferative disorders

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Background

Members of the phosphoinositide 3 kinase-like kinase family of proteins SMG1, ATM, ATR, DNA PK_{CS} and mTOR have well described roles in cellular stress responses including DNA damage and nutrient deprivation - important pathways for cancer survival. SMG1 has a well characterised role in nonsense-mediated decay, a process ensuring the rapid degradation of mRNA containing premature stop codons. In mouse studies, Roberts et al. demonstrated that loss of SMG1 expression led to an increase in occurrence of specific cancers, including mature B cell lymphomas with a predominance of DLBCL. ATM is a tumour suppressor and loss of ATM expression has been linked to lymphoma and leukaemia development as well as altered responses to treatment. Given that a predominant finding in mouse studies was that of haematopoietic cancers, this study will address this spectrum of diseases, in particular CLL and mature B-NHL.

Aims

To assess SMG1 and ATM expression in patients with CLL and B-NHL, analyse downstream pathways which may be important for SMG1 and ATM tumour suppressor activity and determine possible clinical utility for SMG1 and ATM as biomarkers.

Methods

Lymphocytes were isolated from peripheral blood samples (n=78) via Ficoll Paque gradient separation techniques. SMG1, ATM, phosphoAkt, Akt, phosphoP65 and P65 protein levels were measured by Western Blotting and quantitative imaging. Tissue microarrays have been constructed on historic tissue samples and antibody optimisation for immunohistochemical staining is currently underway.

Results and Discussion

Preliminary results demonstrate that absence of SMG1 correlates with higher expression of phosphorylated Akt and hence upregulation of the mTOR pathway (p=0.081). No significant relationship with ATM and phosphorylated Akt was detected (p= 0.2339). By studying SMG1 and ATM biology and generating new knowledge we will identify which pathways are important for tumour formation and which may be targeted as a therapeutic approach to lymphoma and leukaemia in the age of targeted therapies.

166. Comparison of immune changes following emerging therapies for relapsed Chronic Lymphocytic Leukaemia

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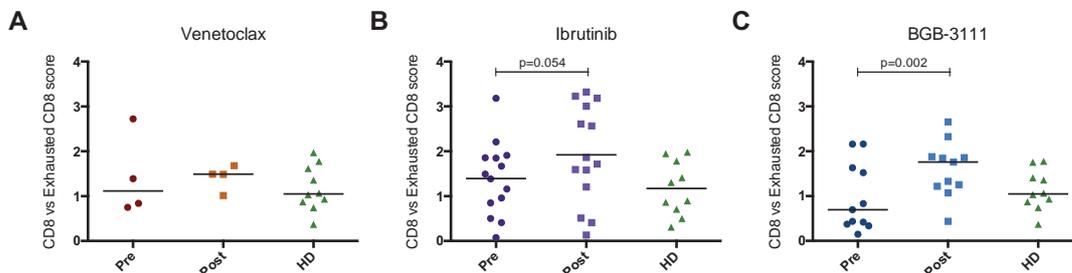
Aim: To quantify the immunological changes observed in Chronic Lymphocytic Leukaemia (CLL) patients after long-term treatment with three emerging therapies.

Method: Peripheral blood mononuclear cells were isolated from CLL patients receiving a BTK inhibitor, Ibrutinib (12 patients) or BGB-3111 (11 patients), or the Bcl-2 inhibitor Venetoclax (6 patients) as part of clinical trials or standard of care at the Peter MacCallum Cancer Centre, Melbourne, Australia. Samples were obtained immediately prior to treatment and after one year of therapy. In addition PBMC from 10 age-matched healthy donors were obtained from the Australian Red Cross Blood Service. Immune profiling was performed by flow cytometry and gene expression was quantified using the nCounter PanCancer immune profiling panel on the Nanostring platform. Statistical analysis was performed using GraphPad Prism and a Wilcoxon or paired t test.

Results: Following Ibrutinib treatment but not BGB-3111, patients showed a significant increase in the proportion of monocytes. Conversely, BGB-3111 treated patients, but not those treated with Ibrutinib had a significant increase in PD-L1 expression on intermediate and non-classical monocytes. Furthermore, in Venetoclax treated patients there was an increase in the frequency of multiple myeloid subsets.

There were no changes in the frequency of T cells or in the CD4:CD8 ratio following treatment, which remained significantly lower than seen in age-matched healthy donors, indicative of T cell dysfunction. Gene expression analysis showed significant increases in the cytotoxic cell score only in patients treated with a BTK inhibitor but not a Bcl-2 inhibitor. However, these cells had increased expression of exhaustion profile genes (Figure 1).

Figure 1: CD8 T cell exhaustion increases after treatment with BTK but not Bcl-2 inhibitors



Conclusion: BTK and Bcl-2 inhibitors have different effects on innate immune subsets. Our results show that while there are improvements in the immunological profile of patients treated with targeted therapies there is persistent immune dysfunction potentially limiting the efficacy of immunotherapy.

167. Combined CML and CLL in Three Patients, including one with 17p deletion

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Aim

The study was performed to review and present three interesting cases of Chronic Myeloid Leukaemia occurring concurrently with Chronic Lymphocytic Leukaemia.

Method

Combined CML and CLL were identified in three patients and cases were reviewed with the WA Cytogenetics Service.

In addition to clinical and demographic information, the results from blood counts, bone marrow studies, flow cytometry, cytogenetics including FISH and molecular testing and monitoring were performed. Via medical records and laboratory information, patient management including the commencement of therapies and response together with clinical progress is obtained. Literature review of CML and CLL with the combination of both is performed.

Result

Three male patients had combined CML and CLL, currently 53, 66 and 66 yo. Prior to diagnosis of CML, all three had a preceding diagnosis of CLL- two 8 years prior, and one two years. Two had received prior Fludarabine. All have received TKIs. None have required subsequent therapy for CLL. One patient has 17p deletion CLL, one has normal CLL cytogenetics. All three currently have satisfactory blood counts and qPCR for CML showing molecular response. All remain alive at 3, 13 and 14 years post initial diagnosis.

Conclusion

The unusual combination of combined CML and CLL in individual patients can be successfully managed by treating each haematological disorder in the usual manner. The excellent control achieved in CML with the TKIs enables satisfactory marrow function to recover in patients with concomitant CLL. Interestingly, the patient with adverse cytogenetics in CLL with 17p deletion remains under observation, with consideration for future therapies such as Ibrutinib to follow. The role for allograft in patients with dual malignancies is uncertain and individualised.

168. Five-year DASISION analysis based on baseline comorbidity and age in newly diagnosed CML-CP patients

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Aim: Baseline comorbidities may influence treatment-related decisions and impact response and survival in patients with chronic myeloid leukaemia (CML). Retrospective analysis of 1-year data from the phase 3 DASISION study reported that baseline comorbidity did not substantially impact outcomes in dasatinib- or imatinib-treated patients. The impact of comorbidities on long-term safety and efficacy were investigated.

Method: Patients in DASISION (NCT00481247) were randomised to receive dasatinib 100 mg/day (N=259) or imatinib 400 mg/day (N=260) and followed for ≥5 years. Treatment-related adverse events (AEs) in ≥10% of patients and response rates were assessed in retrospective groups: 0 vs ≥1 baseline comorbidity, baseline disorder (diabetes mellitus [DM], hepatobiliary disease, hyperlipidaemia, cardiovascular [CV] disorder, or pulmonary condition), or years of age (<46, 46–65, >65).

Result: Within each arm, patients with 0 or ≥1 comorbidity (Table 1) and across age groups, had similar responses by 5 years, although rates were numerically higher for patients with ≥1 vs 0 comorbidities in both arms. The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms; a few AEs, largely grade 1/2, had a ≥2-times higher frequency in patients with ≥1 vs 0 comorbidities (pleural effusion [PE], rash, peripheral oedema, muscle spasms, face oedema, eyelid oedema). The increased incidence of PE in the dasatinib arm was highly associated with increased age. Incidence of PE did not appear to be related to baseline pulmonary comorbidity or smoking history. Baseline CV disorder, DM, or hyperlipidaemia did not significantly increase odds of developing a treatment-related AE (Table 2).

Conclusion: As previously reported, response rates trended in favour of dasatinib vs imatinib and were comparable in patients with 0 or ≥1 comorbidity. Although a few AEs (most grade 1/2) occurred at a higher frequency in patients with ≥1 vs 0 comorbidities, neither baseline comorbidities nor age substantially affected the overall AE incidence at 5 years in patients who were treated with first-line dasatinib or imatinib.

Table 1. Response and safety outcomes by baseline comorbidity

	DASATINIB		IMATINIB	
	Comorbidities			
Responses	≥1 (n = 193)	0 (n = 66)	≥1 (n = 193)	0 (n = 67)
Response by 5 years, n (%)				
CCyR	171 (89)	57 (86)	165 (85)	53 (79)
MMR	148 (77)	49 (74)	130 (67)	36 (54)
MR ^{4,5}	88 (46)	21 (32)	70 (36)	15 (22)

CCyR=complete cytogenetic response; MMR=major molecular response ($BCR-ABL1 \leq 0.1\%$ International Scale [IS]); MR^{4,5}= $BCR-ABL1 \leq 0.0032\%$ IS.

Table 2. Frequent treatment-related AEs by baseline disorder

Baseline disorder	DASATINIB (n=192)		IMATINIB (n=192)	
	Yes (n=43)	No (n=149)	Yes (n=43)	No (n=149)
All AEs, %	79	78	86	77
Baseline DM	Yes (n=18)	No (n=174)	Yes (n=13)	No (n=179)
All AEs, %	78	78	69	80
Baseline hyperlipidaemia	Yes (n=22)	No (n=170)	Yes (n=19)	No (n=173)
All AEs, %	82	78	84	79

169. Retrospective analysis of DASISION: The impact of dose modifications on long-term efficacy in CML-CP patients

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Aim: This 5-year retrospective analysis of DASISION (NCT00481247) was performed to evaluate the impact of dose modifications on efficacy in patients with newly diagnosed chronic myeloid leukaemia in chronic phase (CML-CP) treated with dasatinib or imatinib.

Method: Patients were randomised to receive dasatinib 100 mg/day (N=259) or imatinib 400 mg/day (N=260). Dose reductions/interruptions were specified to manage adverse events (AEs). Up to 2 reductions were allowed: dasatinib 80 mg, then 50 mg; imatinib 300 mg, then 200 mg. Efficacy was assessed retrospectively in patients with or without dose reductions and with dose reductions due to pleural effusion.

Results: For patients treated with dasatinib and imatinib, respectively, 95 (37%) and 44 (17%) had dose reductions and 177 (69%) and 133 (52%) had dose interruptions at any time. Patients on dasatinib versus imatinib maintained higher responses, irrespective of dose reductions, and responses were similar in patients with and without dose reductions in each treatment arm (Table). Many patients saw their responses remain the same or increase following dose reductions (Table).

Conclusion: In this 5-year retrospective analysis of DASISION, responses remained higher for patients on dasatinib versus imatinib with and without dose reductions. Consistent with previous reports, efficacy was not affected by dose reductions for any cause, including pleural effusion. Reducing dasatinib doses to 80 mg or 50 mg were safe and effective treatment options for patients with AEs.

Table.

	Dasatinib	Imatinib	
Molecular response by 5 years, % (95% confidence interval)			
Dose reductions			
None	n=164	n=216	
	MMR	77 (70, 83)	64 (57, 70)
	MR ^{4.5}	44 (36, 52)	31 (25, 38)
Any AE	n=95	n=44	
	MMR	75 (65, 83)	64 (48, 78)
	MR ^{4.5}	39 (29, 50)	41 (26, 57)
Pleural effusion	n=30	n=0	
	MMR	83 (65, 94)	–
	MR ^{4.5}	53 (34, 72)	–
Change in best molecular response from before to after first dose reduction, n (%)			
	n=95	n=44	
Maintained response	31 (33)	9 (20)	
Improved response	40 (42)	17 (39)	
Lost response	8 (8)	3 (7)	
Response changed from MR ^{4.5} to MMR	5 (5)	1 (2)	

IS=International Scale; MMR=major molecular response; MR^{4.5}=BCR-ABL1 ≤0.0032% IS.

170. Factors predicting compliance with Tyrosine Kinase Inhibitors (TKI) in Chronic Myeloid Leukaemia (CML)

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Aim

Identify factors affecting adherence to tyrosine kinase inhibitor (TKI) therapy in chronic myeloid leukemia (CML) in order to target particular populations or individuals with increased education or follow up.

Method

Retrospective chart review of patients with chronic phase CML being followed up at the Princess Alexandra Hospital. Data collection from our prescribing system (CHARM) and electronic medical record included demographics, type and duration of TKI including delays and interruptions. Of the 88 patients reviewed, only 15 patients had adequate data for analysis due to the majority obtaining medications at external pharmacies. Adherence was assessed by calculating days required to complete six month periods of therapy (predefined as 182.5 days) and poor adherence was defined as <90% of therapy completed within this timeframe. Statistical analysis was performed using Fisher's exact test and calculation of odds ratios.

Result

Low numbers limited statistical analysis. Trends toward poor compliance were noted in males and non-Australian born patients, in particular those from Papua New Guinea and the Pacific Islands.

Conclusion

It is known that poor compliance with medication is the most important factor determining disease response and thus outcome. Through identification of at risk patients in our local population, implementation of measures to target them with education and support may help to optimise their outcomes.

171. Effector immune profile of chronic myeloid leukaemia patients on nilotinib and interferon-alpha combination (PINNACLE study)

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Introduction

We investigated immune effector responses serially in newly diagnosed Chronic Myeloid Leukaemia patients treated with nilotinib, combined with pegylated-interferon- α 2b (IFN) after 3 months (mo), within the ALLG CML11 PINNACLE study.

Methods

Samples (n=95) were available 2, 3, 5, 7, 10 and 16 mo from diagnosis. Phenotypic and functional (CD107a) effector immune responses of Natural Killer (NK) cells were characterised by flow cytometry. Functional cytotoxic T-lymphocyte (CTL) responses to leukaemia-associated antigens (LAAs) WT1, BMI-1, PR3 and PRAME were assessed by interferon- γ (IFN- γ)/tumour-necrosis factor- α (TNF- α) FLUROSPOT.

Results

There was a significant increase in cytolytic CD3⁺CD56^{dim}CD16^{br} NK cells after nilotinib (median; diagnosis 20.6% vs 2 mo 38.6%, p=0.002), with a more mature CD57⁺ phenotype and increased activating receptor expression (CD161, NKp46, and NKp30). All patients had *BCR-ABL1* <10% at 3 mo. After IFN, NK cells and CD57⁺ NKs were reduced as early as 24-hours post-IFN. NK degranulation response was unaffected. NKp30/46 expression increased further on combination therapy, with unchanged CD161⁺ expression, and a trend towards more immature CD56^{br}CD16^{-dim} NK cells vs diagnosis. In contrast, NKp44 expression on activated NK cells significantly reduced following nilotinib and combination therapy vs diagnosis. CD3⁺CD56⁺ innate NKT cells increased at 2 mo vs diagnosis, reducing to diagnosis levels after IFN. Low CD62L on CD4⁺/CD8⁺ T cells at diagnosis, indicating impaired immune function, increased after 2 mo nilotinib. IFN- γ and TNF- α CTL responses against all LAAs were observed during combination therapy (predominantly IFN- γ). The most abundant overall were PRAME and PR3.

Conclusions

Nilotinib improves immune effector responses which are blunted at diagnosis. Nilotinib/IFN combination does not further increase NK cell function, but enhances LAA-CTL responses. In contrast to nilotinib only at 2 mo, with IFN, NK cells displayed a less mature, immunoregulatory phenotype (reduced CD57⁺, increased CD56^{br}). Correlation of patient immune responses and clinical outcomes is ongoing.

172. Do we need to exclude BCR-ABL1 positive CML in patients presenting with isolated thrombocytosis? A Western Australia study.

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Background

Isolated thrombocytosis without leucocytosis is a rare presentation of CML, with only few case reports in the literature

Clinicians commonly request peripheral blood BCR-ABL fusion gene testing as the initial investigation of unexplained thrombocytosis, together with screening of JAK2, CALR, MPL mutations.

International recommendations for this practice is divided. The British guidelines recommend BCR-ABL testing only in the “triple negative” scenario or when there are atypical morphological features such as basophilia; whilst the Americans recommend universal upfront BCR-ABL testing.

Our state cytogenetic laboratory receives a high number of BCR-ABL requests to exclude CML in isolated thrombocytosis, with majority of results negative. This approach has been ineffective, time consuming and expensive.

Aim

This study aims to quantify and characterise CML cases presenting with isolated thrombocytosis.

Method

A retrospective study to look at all CML cases diagnosed from 2006-2016 to identify cases with presenting platelet counts $>400 \times 10^9/L$ but white cell counts (WCC) $<11 \times 10^9/L$.

Result

355 cases of CML were diagnosed by our laboratory. 51 cases were excluded as referring pathology services have not provided presenting full blood counts.

4 out of 304 cases (1.3%) were found to have normal WCC but elevated platelet counts (range $462-637 \times 10^9/L$). These cases possessed the common major breakpoints, either B2A2 or B3A2. Additional cytogenetic abnormalities found include loss of Y chromosome and variant translocation of t(9,22), but no ‘major route’ abnormalities was found.

Conclusion

These findings support the deferment of BCR-ABL testing to subsequent bone marrow morphology examination with comprehensive cytogenetic analysis to clarify ‘triple negative’ cases. We are in the process of proposing a state-based diagnostic algorithm which will be presented.

The findings also indicate the possibility of using the commercial kit GeneXpert BCR-ABL Ultra which detects both major breakpoints as an alternative screening method, without concerns of missing cases due to minor breakpoints.

173. Nilotinib vascular and metabolic adverse events may be mitigated with appropriate patient selection and screening

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Aim

To evaluate the incidence of Nilotinib (NIL) related cardiovascular (CV) and metabolic adverse events (AEs), and determine the impact of pre-emptive CV screening.

Background

NIL related CV and metabolic AEs are increasingly recognised in clinical practice. We instituted a pre-emptive CV risk factor assessment and modification for all patients receiving NIL in March 2014. We hypothesised that this intervention would mitigate NIL-related AEs.

Method

Retrospective review of chronic myeloid leukaemia in chronic phase (CML-CP) patients (January 2006-April 2017). Data collected: myocardial (MI) and peripheral vascular occlusive events, new onset dyslipidaemia +/- hyperglycaemia. CV risk assessment: Australian risk calculator and imaging (Doppler ultrasound +/- CT coronary angiography/calcium score).

Results

Identified 52 patients with CML-CP on TKI therapy: median age 51.5 years (range: 21-83), male 62%, median follow-up 51.5 months (4-77mo), first-line TKI therapy 63%. No patient required chemotherapy or allogeneic stem cell transplantation. No CML-related deaths occurred (total: 3). In the 21 (40%) patients who received NIL (71% as first-line therapy), 7 patients discontinued NIL due to intolerance or vascular/metabolic AEs. All were subsequently salvaged with another TKI. None discontinued due to treatment failure. 4 patients (19%) developed vascular AEs (3 MI, 1 peripheral arterial occlusion). 3 patients (14%) developed metabolic dysfunction (1 hyperglycaemia, 2 dyslipidaemia). Median age and time to event in the AE cohort were 48 years (32-71) and 24 months (3 days-61 months) respectively. 75% of patients with vascular AEs were high-risk prior to NIL therapy initiation. All required percutaneous angioplasty or surgical intervention and ceased NIL indefinitely. Since introducing systematic CV risk assessment and modification introduced, no vascular AEs were documented.

Conclusion

NIL-related vascular and metabolic AEs appear common in patients with pre-existing vascular risk and don't necessarily coincide with recent initiation of therapy. Pre-emptive cardiovascular risk factor modification and appropriate patient selection may mitigate risk.

174. Diabetes and Pneumocystis Pneumonia - relevance in Haematological population

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Relation between hyperglycemia and PJP

Diabetes is multisystem disease characterised by hyperglycemia, immune dysregulation and chronic vascular complications. Pneumocystis pneumonia (PJP) is serious but preventable cause of morbidity and mortality in patients treated for haematological Malignancies.

In this setting, PJP prophylaxis is recommended in patients on prolonged high-dose glucocorticoids, defective cellular immunity, acquired immunodeficiency, post bone marrow and solid organ transplant recipients.

Currently, there is no recommendation to include diabetes as risk factor for PJP prophylaxis.

We present two cases of pneumocystis pneumonia at our institution in patients with poorly controlled diabetes and on low risk treatment regimens for PJP.

- A 64-year-old man with stage IIA DLBCL was commenced on R-CHOP21 with curative intent. After 2nd cycle, he required ICU admission for cardiorespiratory failure with severe pneumonia. A diagnosis of PJP was made on endotracheal sample with PJP DNA NAA titre of 5.3×10^5 copies/ml ($>2.5 \times 10^5$ copies is significant). He was treated with Co-trimoxazole and steroids and made complete recovery prior to discharge.
- A 37 year old man with stage IIIA Lymphocyte predominant Hodgkin's Lymphoma on chemotherapy with R-ABVD was admitted with febrile neutropenia after 3rd cycle, and commenced on broad-spectrum antibiotics. The worsening hypoxia and chest radiological changes suggested interstitial lung pathologies like Bleomycin induced fibrosis and PJP. His PJP titre was 2.5×10^4 on induced sputum. He completed recommended PJP treatment and made uneventful recovery.

Both of our patients were poorly controlled Type 2 Diabetics with HbA1c of 13.6 mmol/l (patient A), and 10.9mmol/l (patient B) and not on any PJP prophylaxis based on risk stratifications.

Increasing prevalence of diabetes in patients on intensive curative regime for haematological malignancies raised the issues of prophylaxis in this population. Whether diabetes increases the risk of PJP is a question which needs to be addressed in larger studies.

175. Case series of lymphomatoid granulomatosis at one tertiary hospital in Perth.

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Background

Lymphomatoid granulomatosis (LyG) is a very rare and aggressive Epstein Barr Virus (EBV) associated lymphoproliferative disorder. It typically affects adults in the fourth-sixth decade and is more common in those who are immunosuppressed. EBV infected cells express genes which are considered oncogenic by improving cell survival, driving proliferation and decreasing p53 associated cell death. AIM: To review outcomes at our tertiary institution over a 12 month period.

Method

Through chart and database review we identified three patients with varying sites and grades of disease. RESULTS: The first case involves a 72 year old female that presented with a two month history of dyspnoea and cough. CT scan revealed bilateral, predominantly lower zone nodular infiltrates. Biopsy revealed grade two pulmonary LyG and was managed conservatively. Repeat CT scan two months later showed resolution of the lesions. The second case involves an 86 year old male who presented with acute confusion. MRI brain showed a frontal rim-enhancing lesion with mass effect. Biopsy revealed grade two CNS disease and was managed with interferon. One month later repeat imaging showed 90% reduction in size. The third case involves a 68 year old male with a two month history of cough and B symptoms. Biopsy of the pulmonary lesions revealed grade three disease. He was treated with 6 cycles R-CHOP with end of treatment PET scan confirming progressive disease (Deauville 5).

Conclusion

LyG is a rare EBV associated angio-destructive lymphoid infiltrate. Presentation can be varied and ongoing management is guided by histology and the primary site of disease. Generally speaking, low grade can be managed conservatively whilst high grade is managed similarly to that of diffuse large B cell lymphoma.

176. Methotrexate toxicity and severe social anxiety disorder: supportive care following discharge against medical advice

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Aim

We describe severe idiopathic methotrexate (MTX) toxicity in a 53-year-old man with diffuse large B cell lymphoma and debilitating social anxiety. Unusual supportive care arrangements were employed as standard reversal measures were curtailed by patient-initiated discharge.

Background

The patient was electively admitted for CNS prophylaxis following 6 cycles of R-CHOP chemotherapy at a regional chemotherapy day unit.

Despite unremarkable screening of cardiac function, mental state, creatinine clearance and medication list, the 48-hour serum methotrexate level was 140 times the upper limit of the range anticipated for dose.

High flux haemodialysis and concentrated folinic acid (1500mg/m² IV) were commenced for diuretic-refractory renal failure, circulatory overload and abdominal pain. 2000IU recombinant carboxypeptidase G2 was only partially effective in reducing the serum methotrexate level, (23.9Nm to 8.69Nm) contrary to several previously documented reports supporting its use¹.

Following psychiatric assessment, the patient self-discharged against medical advice from the intensive care unit with advanced renal failure.

Result

Fluid retention, hyperkalaemia and diarrhoea were successfully managed on an outpatient basis. Haematology ward staff titrated diuretics and potassium binding agents via daily phone consultation, assisted by daily pathology testing. Oral folinic acid was continued at 240mg daily throughout. Final serum creatinine was at 311umol/L and the patient survived.

This degree of renal toxicity is a significant outlier from the cohort of 45 previous adults admitted to our institution for high dose MTX infusion.

Conclusions

Despite well documented inter-individual variability in methotrexate metabolism², toxicity after high dose infusion is historically rare in our institution. With limited action against intracellular polyglutamated methotrexate, glucaripidase has a narrow therapeutic window and should be given prior to significant fluid retention where possible.

Careful titration of supportive therapies in a committed outpatient can lead to successful recovery from severe toxicity.

177. Molecular mechanisms of disease progression during ibrutinib therapy in diffuse large B-cell lymphoma-leg type

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Aim: Diffuse large B cell lymphoma-leg type (DLBCL-LT) is one of the well recognised extranodal lymphomas commonly addicted to the B-cell receptor-MYD88 superpathway. We aimed to describe the genomic changes in a patient who progressed through treatment with the BTK inhibitor ibrutinib.

Method: DNA acquired at baseline and at disease progression was extracted and analysed by (i) targeted amplicon sequencing using the Peter MacCallum Cancer Centre (PMCC) lymphoid 27 gene amplicon panel (ii) the PMCC PanHaem panel comprising 313 genes of clinical relevance to haematological malignancy including genome-wide copy number change assessment and IGH translocation detection.

Results/Case description: An 80 year old woman presented with multiply relapsed DLBCL-LT (CD5+, BCL2+, MUM1+, CD10-) after multiple lines of chemoimmunotherapy and radiotherapy. Lymphoid amplicon panel testing of the localised pre-treatment *cutaneous tumour* lesion demonstrated a MYD88 L265P mutation and she was subsequently commenced on ibrutinib. Complete resolution of skin disease was achieved. Despite ongoing skin response, at two months the patient developed progressive inguinal, iliac and para-aortic nodal disease; genomic analysis of the baseline cutaneous tumour sample was compared to the *inguinal lymph node* at progression and revealed acquisition of multiple genomic changes described in table 1. These genomic alterations included multiple aberrations that are expected to bypass BTK inhibition including two *CARD11* activating mutations and a deleterious mutation in NF-κB negative regulator *NFKBIE*. In addition, an IGH-IRF8 translocation was detected (which brings the IRF8 transcription factor under control of the immunoglobulin heavy chain locus), representing a third plausible mechanism contributing to ibrutinib resistance. Several copy number changes occurred in both samples, including 18q amplification which encodes the anti-apoptotic protein BCL2 and also *TNFRSF11A* (encodes a receptor involved in NF-κB activation).

Conclusion: We describe the first case of novel genomic changes of DLBCL-LT that occurred while on ibrutinib providing important mechanistic insights into both pathogenesis and drug resistance.

Table 1: Genomic alterations pre and post ibrutinib

	Pre Ibrutinib	Post Ibrutinib
Variants	MYD88 c.794T>C;p.Leu265Pro	MYD88 c.794T>C;p.Leu265Pro
	CD79B c.586T>C;p.Tyr196His	CD79B c.586T>C;p.Tyr196His
		CARD11 c.367G>T;p.Gly123Cys
		CARD11 c.644A>T;p.Lys215Met
		NFKBIE c.1379G>C;p.Gly460Ala
Copy number changes	Del 4q	Del 4q
	Apparent trisomy 7, 8, 9,10,12	Apparent trisomy 9, 12
	Amplification 18q (BCL2/TNFRSF11A) (~5-6 copies)	Amplification 18q (BCL2/TNFRSF11A) (~5-6 copies)
		Del 2q, Del 4p
Translocation	Not detected	t(14;16) (q32.33;q24.1)

178. Treatment outcomes for Primary Thyroid Lymphoma: Hospital Ampang's experience

Hon S¹

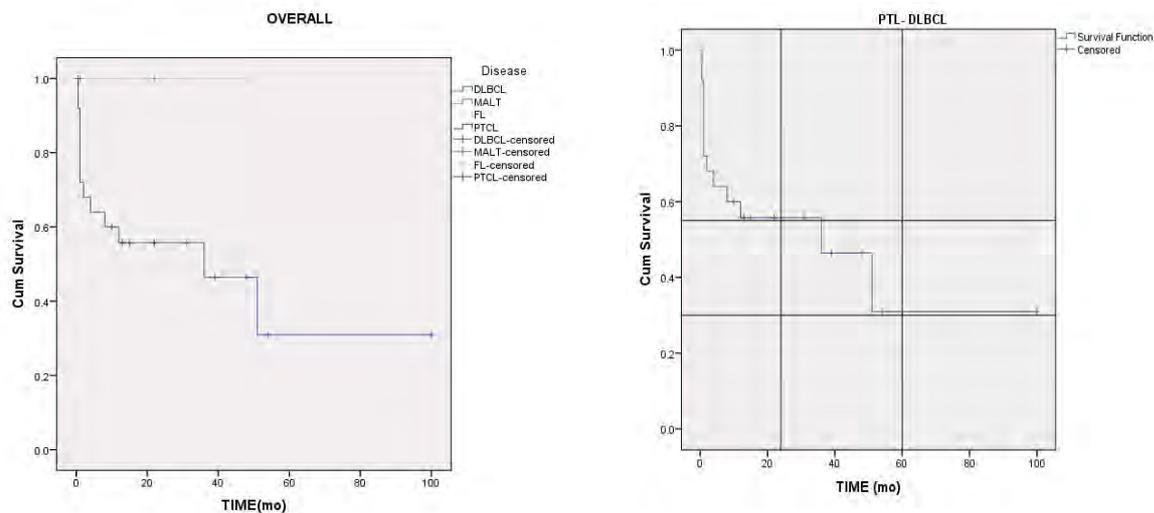
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Purpose: Primary thyroid lymphoma (PTL) is a rare disease, accounts for about 5% of all thyroid malignancies and about 1% to 2% of all extranodal lymphoma. We aim to analyse the treatment outcome in PTL patients in Hospital Ampang.

Methods: This is a retrospective analytical study obtained from Hospital Ampang electronic database from year 2007 to 2016. A total of 29 patients with PTL were identified. Diagnosis was confirmed by HPE from thru-cut biopsy (n=11) or thyroidectomy (n=18). Treatment modality and outcomes were analysed.

Results: The median follow-up was 13 months (range 1 to 100 months). The median age of diagnosis was 62 years (range 32 to 100 years), and 17 (59%) were females. 25 (87%) were DLBCL, 2 (7%) were MALT, 1 (3%) follicular lymphoma (FL) and 1 (3%) peripheral T cell lymphoma (PTCL). 15 (52%) had RIPI score 2 or more at diagnosis. 3 (10%) were treated with surgery alone, 11 (38%) with chemotherapy alone, 14 (49%) with both total thyroidectomy and chemotherapy, and 1 (3%) was treated with combination of surgery plus chemo-radiotherapy. There were no death or relapse among MALT and follicular lymphoma patients. Among DLBCL cohort, the overall survival (OS) rate was 55% at 24 months and 30% at 60 months respectively. RIPI score was a significant prognostic factor for OS. Total thyroidectomy with chemotherapy in PTL showed a survival rate of 60% at 5 years. However, different treatment modalities in DLBCL didn't significantly affect the treatment outcomes.

Conclusion: The optimal treatment of PTL remains controversial, and treatment approach mainly according to PTL subtypes. From our cohort, MALT and FL subtype showed favourable outcome, whereas for aggressive subtype like DLBCL, multimodality treatment is essential and chemo-immunotherapy still the mainstay of treatment. Surgery should be reserved for selected cases only.



179. Idiopathic Multicentric Castleman's Disease with Normal Interleukin-6 Levels

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Multicentric Castleman's disease (MCD) is a rare clinicopathological disorder characterised by systemic lymphadenopathy, B-symptoms and classical histopathological lymph node findings. Hypercytokinemia, particularly interleukin-6 (IL-6), secondary to human herpes virus-8 (HHV-8) infection is becoming increasingly recognised as essential to MCD pathogenesis. However, MCD can occur independent of HHV-8 infection in an entity termed idiopathic MCD (iMCD). Rarely, iMCD can occur in the absence of elevated IL-6 levels thus suggesting that other cytokines and pathways also contribute to the pathogenesis of iMCD.

We report an uncommon case of iMCD with normal IL-6 levels in a 30-year old previously well lady. She presented with a 9-month history of progressive multifocal lymphadenopathy associated with night sweats, fevers and fatigue in the absence of anorexia or weight loss. Physical examination revealed mobile and non-tender lymphadenopathy in the cervical, axillary and inguinal regions. Laboratory investigations were consistent with features of systemic inflammation. Screening autoantibodies, particularly for systemic lupus erythematosus, was negative. Thoracic and abdominal imaging demonstrated multiple enlarged lymph nodes above and below the diaphragm.

Excisional biopsy of a right inguinal lymph node revealed a dominant interfollicular expansion with increased number of follicles and an 'onion skin' concentric arrangement of the mantle zone, consistent with Castleman's disease. Bone marrow biopsy performed also revealed marrow involvement. Serology for human immunodeficiency virus and HHV-8 subsequently returned negative. These findings in conjunction with the lymph node features lead to a final diagnosis of iMCD. Surprisingly, her IL-6 levels was also found to be normal.

The patient was treated with a 3-month course of high dose corticosteroid therapy with resultant disease remission beyond two years follow up.

180. Maximising yield of peripheral blood flow cytometry for chronic lymphoproliferative disorders

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Aim

Flow cytometry is used in the diagnosis of haematological diseases including chronic lymphoproliferative disorders and is often requested by clinicians for various indications. This audit aims to ascertain the real-world indications for peripheral blood flow cytometry and which of these indications are associated with higher diagnostic yields.

Method

All peripheral blood flow cytometry requests for chronic lymphoproliferative disorders panel from 1 January 2014 to 31 December 2014 were identified using the laboratory information system. Patients with known existing lymphoproliferative disorders were excluded. Data including patient demographics, specialty of requestor, lymphocyte count and blood film report (if available), indications for tests and subsequent diagnosis were collected.

Result

A total of 185 patients with median age of 60 (19-90 years), were analysed. There was a slight female predominance (n=96, 51.9%). The main indications for testing (as provided on the request) were peripheral blood lymphocytosis, defined as lymphocyte count $>4 \times 10^9/L$, (39/185; 21.1%) followed by cytopenias (34/185; 18.4%). The median lymphocyte count at time of testing was $2.1 \times 10^9/L$ (0.2 – $197.3 \times 10^9/L$). The main requestor was the Haematology Unit (n=109; 58.9%) although the diagnostic yield of their requests were not significantly better than other units combined (16.5% vs 13.2%, p=0.49). Other requestors include Nephrology (n=21; 11.3%), General Medicine (n=12; 6.5%) and Infectious Diseases (n=8; 4.3%). Twenty-eight (15.1%) of the tests detected clonal lymphoproliferative disorder, all of which were B-cell in lineage. Factors that significantly improved the diagnostic yield of testing were the presence of atypical lymphocytes on the blood film and lymphocytosis (p<0.01). Constitutional symptoms and cytopenias were not found to influence the diagnostic yield.

Conclusion

Peripheral blood flow cytometry is a useful tool when used in the appropriate clinical setting. Rationalisation of testing is important to reduce the futility of testing and unnecessary health costs.

181. Augmented ICE in Patients with Poor-Risk Refractory and Relapsed Lymphoma

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Aim

Salvage chemotherapy followed by autograft is standard treatment for relapsed/refractory lymphoma. Response to conventional salvage is poor in patients refractory to induction or relapsing within 12 months of remission and justify alternative approaches with new agents or intensified chemotherapy. We report our experience with augmented ICE (10,000mg/m² ifosfamide[vs standard:5mg/m²], carboplatin AUC5 and 600mg/m² etoposide[vs standard:300mg/m²]) in this group.

Method

Between 2011-2016, 17 patients with diffuse large B-cell (DLBCL) and Hodgkin lymphoma with refractory disease or relapse within 12 months, or T-cell lymphoma with advanced IPI at relapse within 24 months received two cycles of inpatient augmented ICE with G-CSF support. Chemosensitive patients proceeded to stem cell collection and autograft.

Results

The table below describes demographics and outcome. Sixteen of 17 patients completed both cycles. Median number CD34x10⁶/kg collected was 7.1(12 patients post-cycle one, 5 post-cycle two). Median time between cycles was 20 days. Median days in hospital were 12 (first cycle) and 10.5 (second cycle).Overall response rate and transplantation rate was 94%

	DLBCL	Hodgkin	T-cell Lymphoma
Number of patients (n)	11	5	1
Median age	51	23	61
Response rate	10 (91%) (6 CR,4 PR)	5 (100%) (3 CR,2 PR)	1 (100%) (CR)
Transplant	10	5	1
Overall survival (median follow up 48 months)	55%	100%	100%

Treatment toxicities included grade 4 haematological (febrile neutropaenia in 10 in first cycle and 8 in second cycle; thrombocytopenia and anaemia requiring transfusion in 12 and 10 in first cycle, and 8 and 11 in second cycle), reversible ifosfamide encephalopathy in 3 patients, haemorrhagic cystitis and ifosfamide nephrotoxicity in one patient each. Two patients required dose reduction between cycles.

Conclusion

Augmented ICE is associated with a high response rate in poor risk lymphoma at the expense of moderate toxicity. Whether this approach of chemo-intensification in the era of new antibody and immunotherapy remains relevant is, however, controversial.

182. Unusual Spontaneous Regression of Relapsed Cutaneous Follicular Lymphoma (FL)

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Spontaneous regression of low grade B-cell non-Hodgkin lymphoma (NHL) is reported but remains uncommon. We report a case of a 62-year-old man with spontaneous regression of predominantly cutaneous and non-bulky nodal multiply relapsed follicular lymphoma.

Our patient initially presented with stage 4 follicular lymphoma in July 2012 with cutaneous, nodal and bone marrow involvement with a FLIPI score of 3. She was treated with six cycles of Rituximab-CHOP with partial response on positron emission tomography restaging. Extensive cutaneous progression 12 months later necessitated salvage therapy with six cycles of Rituximab and Bendamustine (BR) with complete metabolic response at completion. No maintenance rituximab was given.

Relapsed cutaneous involvement of extremities, chest wall and back 4 months post-BR was confirmed histological (grade 3a) FL with nodal involvement on PET scan. He developed asymptomatic severe neutropenia during subsequent follow up likely due to prior Rituximab. Given the non-bulky asymptomatic involvement, he was carefully observed where upon all of his cutaneous and nodal lesions spontaneously disappeared 18 months later and this was confirmed on PET. He remains in ongoing remission during his latest review in July 2017.

Spontaneous remission from relapsed follicular lymphoma post immuno-chemotherapy is rare and the mechanism is unclear, but possibly due to immune reconstitution and recovery of tumour reactive T-cell immunity such as the PD-1 pathway.

183. Brentuximab vedotin in relapsed/refractory Hodgkin lymphoma as a bridge to transplant: a real-world, single-centre experience

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Aim

Brentuximab vedotin (BV), an antibody-drug conjugate targeting CD30,ⁱ induces overall response rates (ORR) of 58-80% in Hodgkin Lymphoma (HL) within 3-4 cycles.^{ii,iii} Its role as salvage therapy in relapsed/refractory patients following stem cell transplantation (SCT) is well established; increasing data suggests BV may also be used as a bridge to high dose therapy (HDT) and SCT.^{2,iv,v} This study reports a single-centre experience of the efficacy and tolerability of BV prior to SCT.

Method

A retrospective analysis of outcomes in patients with relapsed/refractory HL managed with BV at Flinders Medical Centre was conducted. Response was assessed by positron emission tomography (PET) scanning.

Results

8 patients (age 26-39) received BV; 3 patients with primary refractory HL, 5 patients with refractory disease at relapse. Patients had received a median of two prior chemotherapy regimens (range 2-5).

A median of 3.5 cycles of BV were administered (range 2-4), with response assessed after a median of 3 cycles. The ORR was 100%; 4 patients (50%) achieved a complete response (CR), 4 patients (50%) achieved a partial response (PR). 6 patients received HDT-SCT (5 autologous, 1 allogeneic); 4/6 achieved CR prior to transplant. Two patients had previously been deemed unfit for transplantation due to poor performance status; disease control by BV improved their clinical status and allowed consolidation by HDT-SCT. All patients achieved CR post transplant, and 5/6 transplanted patients remain in CR with a median follow up of 2 years. One patient with relapsed disease post allograft recommenced BV and achieved a PR.

Conclusion

BV is well tolerated, with deep responses achieved within a short time frame of 2-4 cycles. During BV therapy patients maintained or improved their performance status, allowing consolidation with HDT-SCT and resulting in a durable CR. BV is thus a viable therapeutic option as a bridge to transplant, even in candidates not initially considered as suitable for HDT based on performance status.

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184. Ten years of Mantle Cell Lymphoma at the Royal Hobart Hospital.

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Aim

Mantle cell lymphoma (MCL) is a rare and aggressive lymphoma. Treatment options are often limited by patient comorbidities and a lack of consensus regarding induction regimens. We aim to review our local experience.

Method

We performed a retrospective analysis of patients treated for MCL at The Royal Hobart Hospital (RHH). Patients were identified by data extraction from our electronic pathology database (Kestral). Patient demographics, treatment and outcomes were collated from the electronic medical record.

Result

Ten patients were treated for MCL at The RHH from 2005 to 2015. This suggests the annual incidence of MCL in Southern Tasmania is approximately 0.5 per 100,000. This is in keeping with the reported incidence seen in western countries¹. Our patient cohort demonstrated a marked male predominance (9:1). The median age of patients was 67 years. A wide variety of induction regimens were utilised and two patients were initially treated with local radiotherapy alone. Four patients (40%) underwent myeloablative therapy and autologous stem cell transplant. The most common reason for transplant ineligibility was advanced age at diagnosis. After a median of 20 months of follow up (range 3-141 months) there were three deaths were attributable to progressive disease and one attributable to treatment related mortality.

Conclusion

Our local experience is similar to that of other centers¹. In light of the varied induction approaches, future studies should focus on determining risk-adapted standard of care for induction treatment of transplant eligible and ineligible patients¹⁻³.

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185. A rare cause of seizures.

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Aim

To highlight the difficulties in diagnosis and management of intravascular large B-cell lymphoma (IVLBCL) with CNS involvement.

Case

We report on a case of a 52 year old gentleman presenting to the Royal Hobart Hospital in status epilepticus. This is on a background of excessive alcohol use, malnutrition and Crohn's disease. Following control of seizure activity, clinical examination revealed a marked expressive dysphasia, dysphagia and left hemiparesis. Initial evaluation with MRI imaging revealed extensive areas of intraparenchymal hyperintensity, microhaemorrhages and a right frontal lobe mass lesion extending into the lateral ventricle. Histological evaluation of a brain biopsy confirmed presence of an intravascular population of large CD20 positive cells in keeping with IVLBCL. Initial treatment with Rituximab and high dose Methotrexate was chosen due to poor performance status and low body weight of 38kg.

Progress

Following one cycle of chemotherapy, the patient made good neurological recovery-progressing from hoist transfer to mobilising 20 meters with minimal assistance. Further chemotherapy will include R-CHOP and high-dose methotrexate.

Conclusion

IVLBCL with CNS involvement is a rare lymphoma with a high mortality rate. Many patients are diagnosed posthumously or are too unwell at time of diagnosis to tolerate chemotherapy¹. There is currently no consensus regarding a chemotherapy regimen. This case highlights the tolerability of Rituximab and Methotrexate, and its use as a bridge to more definitive therapy.

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186. Tattoo pigment-induced granulomatous lymphadenopathy mimicking lymphoma

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A 30-year-old female presented with a two-week history of bilateral axillary lumps. She had no systemic symptoms and was otherwise well. Examination demonstrated numerous rubbery non-tethered nodes up to 1.5 cm in diameter in both axillae. Positron emission technology-computed tomography revealed multiple enlarged, markedly glucose avid lymph nodes in the axillary, hilar and mediastinal areas, with a peak SUV of 17.7 and maximal size of 13x23 mm, thought to be consistent with lymphoma.

A left axillary node was excised and found to be enlarged and black. Microscopically, the nodal architecture was replaced by well-formed epithelioid granulomas with scattered multinucleate giant cells. Collections of black pigment-laden macrophages were noted within the paracortex. Organism stains for mycobacteria and fungi, and immunohistochemistry for malignant disorders were negative. Flow cytometry did not detect an abnormal lymphoid population. The patient had a large decorative tattoo covering her back which had been inked 15 years previously. A diagnosis of granulomatous lymphadenitis was made, likely a hypersensitivity reaction to tattoo pigment. In retrospect, the patient recalled that her tattoos became transiently pruritic and raised in areas for a few days each month. She has been monitored clinically and her lymphadenopathy has reduced.

A small number of cases of delayed tattoo pigment lymphadenopathy have been reported, usually localised to one lymph node region. The case we describe is unique in the clinical and radiological presentation of metabolically active, widespread lymphadenopathy which mimicked lymphoma. It suggests tattoo pigment lymphadenopathy as a potential differential diagnosis for lymphoma and highlights the importance of a careful tattoo history and physical examination

187. Outcome of Mantle cell lymphoma patients in a tertiary teaching institute

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Aim

Mantle cell lymphoma (MCL) is an uncommon non-Hodgkin lymphoma for which outcome remains quite variable. The aim of this study is to analyse clinical characteristics of MCL patients in regard to the outcome in a tertiary teaching institute.

Method

The study was a retrospective clinical audit of all patients diagnosed with MCL at the Canberra Hospital between July 2011 and June 2016. Demographic information, disease characteristics, treatment protocols and survival were studied.

Results

A total of 36 patients received treatment for MCL during the study period. Males were over represented (88.6%) and the mean age of diagnosis was 66.5 years. The majority of patients (88.2%) were stage III or IV at diagnosis. The Mantle Cell Lymphoma International Prognostic Index (MIPI) was calculated for 28 patients, and 14.3%, 25% and 60.7% patients were in low, intermediate and high risk, respectively. Induction chemotherapy regimens are detailed in Table 1. Complete remission was achieved in 93.1% of patients and of these, 70.4% had consolidation with autologous stem cell transplantation. Progression free survival (PFS) was calculated for 20 patients where information was available with a mean of 67.9 months (95% confidence interval 45.29-90.6 months). Survival based on MIPI score is displayed in Image 1. Patients treated with Nordic protocol CHOP including autologous transplantation achieved better progression free survival.

Conclusion

As patients with MCL are quite diverse as far as outcome is concerned. In this population patients treated in the Nordic protocol followed by autologous transplant had a slightly better outcome. It needs further collaborative approach to better understand this condition.

	Number (%)
CHOP/R-CHOP	12 (38.7)
Nordic protocol	12 (38.7)
R-hyper-CVAD	2 (6.45)
R-CHOP + FMD	1 (3.2)
R-Maxi-CHOP + FCR	1 (3.2)
Other	3 (9.68)

188. High-dose methotrexate protocols and survival outcomes in primary central nervous system lymphoma (PCNSL)

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Aim

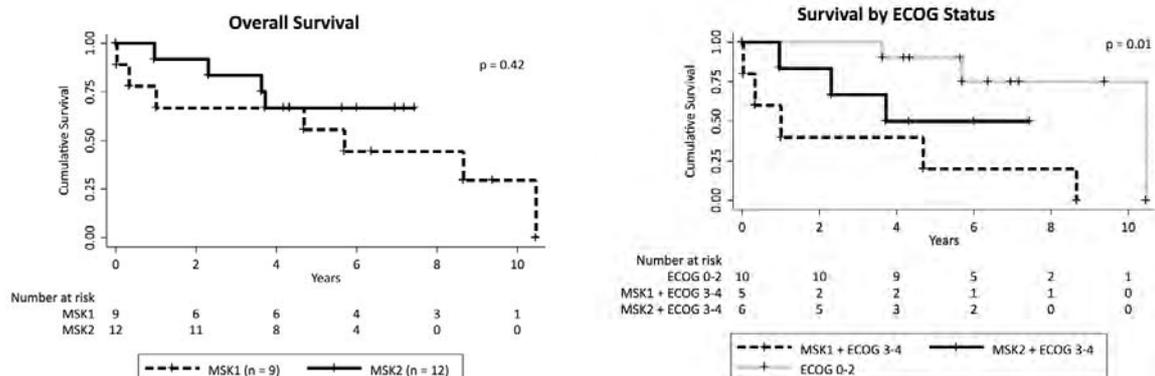
To retrospectively evaluate the clinical impact of modifying a methotrexate, procarbazine and vincristine protocol (MSK1) to incorporate more methotrexate, rituximab (CD20 antibody) and reduced radiation doses (MSK2).

Method

Twenty-one immunocompetent patients at St Vincent's Hospital Melbourne with newly diagnosed PCNSL received two sequential regimens: MSK1 (2003 to 2011, n = 9) or MSK2 (2009 to 2012, n = 12). The median ages were 74 (MSK1, 23-81) and 63 (MSK2, 52-78, p = 0.21). Both groups were similar in performance status and prognostic scores. Outcomes were calculated from the first day of chemotherapy; statistical analysis was conducted using the Fisher exact test, Mann-Whitney U test and Kaplan-Meier as appropriate.

Result

The median follow-up for surviving patients was 7.88 years (MSK1) and 5.82 years (MSK2). Complete response was achieved in 44% (MSK1) and 67% (MSK2, p = 0.40). There was no significant difference in PFS or OS: MSK1 (PFS = 3.75 years, OS = 5.70 years) and MSK2 (median values not reached, PFS: p = 0.29, OS: p = 0.42). On univariate analysis, age > 75, poor performance status (ECOG 3-4) and methotrexate dose were significantly associated with inferior OS. Stratification of patients by age and ECOG showed that the poor outcome of older patients was entirely explained by their inferior ECOG status. Patients with good ECOG status (0-2) had favourable PFS (2-year: 80%) and OS (2 year: 90%, p = 0.92 comparing protocols) irrespective of the regimen received. In contrast, those with an ECOG 3-4 showed a trend of improved survival with MSK2 relative to MSK1 (2-year OS: 83% vs 40%; p = 0.30). The incidence of delayed neurotoxicity was 24% (p > 0.05).



Conclusion

Performance status greatly influences survival outcomes. Our results support the ongoing use of MSK2 as the standard regimen especially in PCNSL patients with a poor performance status (ECOG 3-4).

189. Nivolumab for relapsed/refractory classical Hodgkin lymphoma after autologous transplant: CheckMate 205 full extended follow-up results

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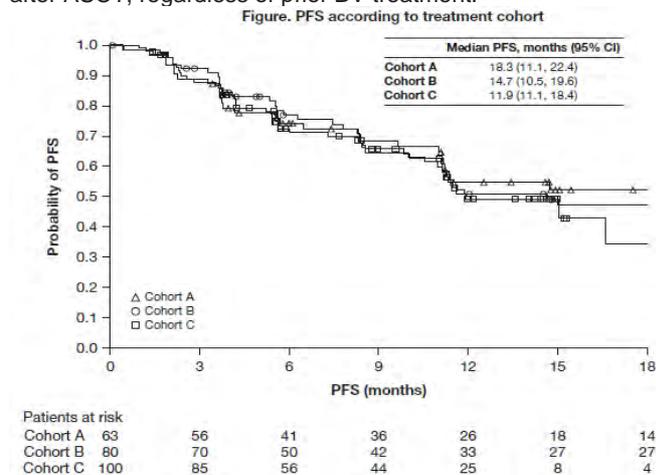
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Aim: Nivolumab is indicated for relapsed/refractory classical Hodgkin lymphoma (R/R-cHL) after autologous stem cell transplant (ASCT) and brentuximab vedotin (BV). The phase 2 CheckMate 205 trial (NCT02181738) of nivolumab for R/R-cHL patients after ASCT initially demonstrated high objective response rates (ORR), encouraging duration of response (DOR), and acceptable safety. We report extended follow-up data.

Method: Patients were enrolled into 1 of 3 cohorts (A: BV-naïve; B: BV after ASCT; C: BV before and/or after ASCT) and received nivolumab 3 mg/kg Q2W until progression/unacceptable toxicity. Cohort C patients with 1-year complete response (CR) discontinued nivolumab and could resume at relapse. Primary endpoint: ORR per Independent Radiology Review Committee. Additional endpoints: DOR, progression-free survival (PFS), overall survival (OS), safety.

Results: Of 243 patients treated, 63 were BV-naïve (Cohort A), 80 had BV after ASCT (B), and 100 had BV before and/or after ASCT (C). Median age: 34 years. At Dec-2016 database lock, median follow-up was 19 (Cohort A), 23 (B), and 16 months (C); 40% remained on treatment. ORR was 65% (Cohort A), 68% (B), and 73% (C), with CR in 29% (A), 13% (B), and 12% (C). Median DOR was 20 (Cohort A), 16 (B), and 15 months (C). For patients with CR, DOR was 20 months (Cohort A) and ≥15 months (B and C); for patients with partial response (PR), DOR was 17 and ≥11 months, respectively. Figure shows PFS. Median PFS was ≥17, ≥15, and ≥9 months for patients with CR, PR, and stable disease. Median OS was not reached. The most common drug-related adverse events were fatigue (23%), diarrhoea (15%), and infusion reactions (14%); most common drug-related serious adverse events were infusion reactions (2%) and pneumonitis (1%).

Conclusion: Extended follow-up demonstrated high, durable CRs and PRs to nivolumab in R/R-cHL patients after ASCT, regardless of prior BV treatment.



190. Nivolumab in advanced relapsed/refractory lymphoma. the Western Australian Experience

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Background: Hodgkin's Lymphoma (HL) is a neoplastic disease of lymphoid origin that can be cured in more than 75% of patients with frontline chemotherapy. Relapsed/refractory patients might be salvaged by high-dose therapy and autologous stem cell transplantation (ASCT). There is no standard of care for the treatment of relapsed/refractory HD once they failed ASCT. Allogenic bone marrow transplantation is recommended in young patients, with about 30-40% chance of long survival. Alternately, novel agent as Brentuximab and Nivolumab can be used.

Aim: To investigate the efficacy of Nivolumab in the treatment of multiple relapsed/refractory HD

Method: From 2015 to 2017 3 patients in Western Australia were treated at our centre with Nivolumab at the dosage of 3 mg/kg of body weight once every 2 weeks without premedication until a complete remission, disease progression or unacceptable toxicity as assessed by the investigators.

Results: Patients characteristics are described in Table 1. Patient n1 showed a poor compliance at Nivolumab treatment due his mental status. He suffered also bipolar affective disorder, revised to paranoid schizophrenia. After an initial response, a disease progression occurred. Patient n2 received 24 cycles of Nivolumab obtaining a complete remission of his disease and now, he is in good clinical condition. Patient n3 received 49 doses of Nivolumab without side effects. After an initial PET/CT that showed a CR, a slow disease progress was registered. Now the patient is in PR with very good clinical response.

Conclusion: Although allogenic transplant is indicated for patient with relapsed or refractory HD, only 30%-40% of patient achieve a long survival. Nivolumab has reported therapeutic activity with acceptable toxicity in this subset of patients. Further investigation are necessary to established which of these treatments, or if a combination of both, could increase long term survival in heavily treated relapsed/refractory HD.

Table 1: Characteristics of patients and response to Nivolumab

	PATIENTS		
	1	2	3
Disease	Nodular sclerotic classic HD	Nodular sclerotic classic HD	Classic HD
Date diagnosis	March 2013	Sept 2014	Apr 2012
Age	18	16	27
Initial therapy	ABVD¹ x 6	COPDAC² x 4	ABVD x 6
BEAM-ASCT	YES	YES	YES
N° chemotherapy lines	3	2	6
N° Brentuximab vedotin cycles	3	0	0
N° Nivolumab cycles	7	24	49
Response to Nivolumab	PD	CR	PR
Side effects to Nivolumab	None	Hypothyroidis Arthalgias	None

191. A Rare Case of Intravascular Large B Cell Lymphoma with Bone Marrow Involvement: A Case Report

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Intravascular large B cell lymphoma is a rare type of an extranodal large B cell lymphoma characterised by the selective growth of lymphoma in the lumina of medium and small blood vessels including capillaries. It is an aggressive lymphoma with a poor prognosis.¹

Our patient, a 66 year old previously well man presented with right upper quadrant abdominal pain and B symptoms over 3 weeks. On admission he had postural hypotension. Skin rashes, palpable lymphadenopathy or hepatosplenomegaly were not detectable but, mild splenomegaly was detected by an abdominal CT scan. He had marked hyponatremia with low urine sodium and elevated liver enzymes. Main haematological findings were marked thrombocytopenia ($20-40 \times 10^9/L$) with a leucoerythroblastic blood picture, a small population of clonal B lymphocytes with CD5+, CD19+, CD20+, CD10-, CD200- in the peripheral blood and high LDH (1788 u/L). His marrow biopsy showed cohesive clusters of large immature malignant cells with moulding in the aspirate which was misleading for a metastatic deposit. However, flow cytometry showed a small population of cells with a similar phenotype to the peripheral blood. The trephine biopsy showed CD45 and CD20 positive large atypical cells with in vascular lumina with vascular endothelium highlighted by CD34, which is consistent with intravascular B cell lymphoma

He unfortunately developed hospital acquired pneumonia, clinically deteriorated and needed ICU care for non invasive ventilation and for inotropic support to maintain blood pressure. However, he refused invasive ventilation and expired after 11 days of admission.



Figure 1- Bone
Marrow aspirate
Giemsa stain

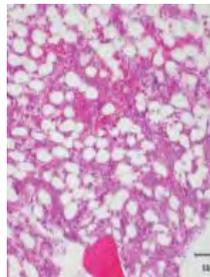


Figure 2 –Trephine
biopsy
Haematoxylin and Eosin

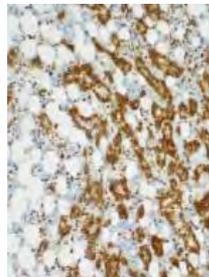


Figure 3– Trephine
biopsy CD20

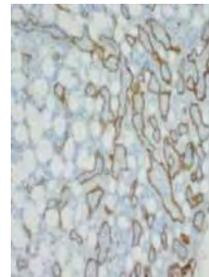


Figure 4 –Trephine
biopsy CD34

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192. Outpatient administration of infusional lymphoma protocol DA-EPOCH to increase inpatient bed access

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Aim:

To develop and pilot an outpatient (OP) protocol for the 96-hour infusional Dose Adjusted (DA-)EPOCH protocol to improve inpatient (IP) bed access.

Background

Pressure on IP beds is increasing. DA-EPOCH (etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone) is increasingly employed for patients with high-risk aggressive lymphomas, and includes a 96-hour continuous infusion, traditionally given as an IP. With increasing pressures on bed access OP administration and ambulatory infusions was considered.

Method

Stability of the agents in ambulatory infusion devices was established. Eligibility criteria for OP administration were identified and a protocol developed. The Day Therapy unit was engaged for scheduling for OP administration. A cohort of patients were transferred to OP therapy and the viability and acceptability of the OP protocol assessed in a pilot program.

Results

Drug stability within an ambulatory infusion device (Baxter Infusor®) was established. Two 48-hour infusions were chosen to accommodate protocol specified dose increases. To date four patients have been treated as OP with a total of seven cycles administered. Three patients commenced therapy as IP before transferring to the OP program, one patient commenced OP therapy from cycle one. No increase in adverse effects has been observed in the OP group. One cycle was complicated by an occluded venous line resulting in approximately 24 hours of no drug infusing, this was identified at the 48 hour point and the infusion extended.

Conclusion

The pilot of the OP DA-EPOCH protocol has been well tolerated with only one infusion issue. Patient acceptance and feedback has been positive and the release of five IP bed days per OP cycle allowed increased access to highly demanded IP beds. The pilot program will continue with planned expansion in the number of patients it can be offered to.

193. The Lymphoma and Related Diseases Registry: Improving the quality of care for lymphoma patients

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Aim

The Lymphoma and Related Diseases Registry (LaRDR), established in June 2016, aims to improve the quality of care of lymphoma through providing systematic data collection on patterns of treatment and clinical outcomes of lymphoma patients.

Method

All incident Hodgkin and non-Hodgkin lymphoma and related disease cases at participating sites are included in the registry. Baseline and 6, 12 & 24 month follow-up visits will provide data on diagnosis, co-morbidities, therapy, supportive care, and outcomes which is entered via a secure web-based database. The registry, currently collecting data from pilot sites in Australia, will expand to be representative of sites across Australia and New Zealand. Hospital reports, providing de-identified risk-adjusted outcome data, will allow individual sites to benchmark performance with comparable health services and nationally. An ANZ multidisciplinary Steering Committee oversees registry operational and research activities.

Results

Ethics approval has been granted at 8 sites and data entry has commenced at 5 pilot sites. To date 161 patients have been registered: median age was 67y, 53% were male and mean BMI was 29kg/m² (95%CI 25.7 to 32.7kg/m²). Initial analysis of diagnostic data indicated a diverse range of lymphomas recorded; the 161 cases were made up of 14 different types of B-cell non-Hodgkin lymphomas, 6 types of T/NK-cell non-Hodgkin lymphomas and 4 types of Hodgkin lymphomas.

Conclusion

With over 6000 new cases expected to cause 1400+ deaths in Australia in 2017, lymphoma represents a significant burden of disease to the community and to health budgets. By monitoring lymphoma treatment and outcomes, the LaRDR will provide valuable research and clinical quality improvement data. Current work includes site expansion, and establishment of a Pathology Review Sub-committee to validate collected data. Future work includes data validation, preparation of hospital reports and research outputs such as health economics analyses. Further information is available at www.lardr.org.

Keywords: Lymphoma, Registries, Quality of care

Conflict of interest: This research is supported by Takeda, Roche, Celgene, Janssen, Novartis and Bristol-Myers Squibb. The companies had no role in analysing the data or preparing the abstract.

194. Bendamustine Rituximab in elderly patients with Waldenstrom Macroglobulinemia showing early benefit with fewer treatment cycles.

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Aim

Bendamustine and Rituximab (B-R) is the preferred first line treatment option for Waldenstrom Macroglobulinemia (WM) in Australia. The recommended regime of 6, twenty eight day cycles of B-R is reported to be well tolerated in the elderly and/or in those who have poor performance status. If intolerant, the dose or number of cycles may be reduced to 4. We observed a small cohort of 4 elderly patients with WM who were intolerant to more than 3 cycles of B-R therapy, though had shown good early response.

Method

Analysis was undertaken of 4 patients treated at our institution for WM, aged 68 years of age and older. All patients had received a combination of B-R and Rituximab (R) alone. Patient medical records were reviewed and included review of haemoglobin, monoclonal IgM and monoclonal paraprotein prior to commencement of therapy, earliest results from September 2016, and again in June 2017 when all patients had completed therapy.

Results

One patient tolerated 3 cycles of B-R without delay in therapy. The other three patients received 2 cycles of B-R and subsequent cycles, of B-R or R, were delayed due to prolonged cytopenias. Two of these three patients required Granulocyte-Colony Stimulating Factor and one patient required red blood cell transfusion support and was hospitalised twice for neutropenic sepsis. All patients received between 3 to 5 cycles of therapy incorporating B-R and single agent R. To date all patients have had an improvement in haemoglobin, and reduction in monoclonal IgM protein and monoclonal paraprotein.

Conclusion

Our experience shows B-R causes significant dose limiting toxicity in the elderly, however even without long term data, with 3 or less cycles of therapy, B-R shows activity in WM.

196. WhiMSICAL (Waldenstrom's Macroglobulinemia Study Involving CART-wheel) update: empowering patients internationally to contribute data for research

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Aim

Waldenström's Macroglobulinemia (WM), a rare cancer with easily trackable disease parameters, is difficult to study in large trials. Having demonstrated advances in research and survival,¹⁻³ patient-derived data is an attractive option to gain breadth of knowledge in WM.

This study utilised www.cart-wheel.org, an ethically-approved, online rare cancer database for patient-derived data, and WM patients' digital connectedness to develop an expanding patient-derived dataset, providing foundation for hypothesis generation around WM patient reported outcomes and improving our understanding of this rare disease.

Method

An HREC-approved, WM-specific extension to www.cart-wheel.org's questionnaire, developed by clinician and patient investigators, went online June 2016. Participants provide data on symptoms, pathology results, treatments, their tolerance and how they were accessed. International promotion by the International WM Foundation was undertaken following development of online consent and demonstration of feasibility locally.

Results

Local recruitment demonstrated initial project feasibility with 69 participants. Following international promotion in May 2017, 83 further participants were recruited in 6 weeks. The 152 participants were predominantly from USA (40%), Australia (33%), Canada (9%) and UK (8%), with median age 67 years (44-85) and male predominance (65%). Fatigue was the most common symptom listed on presentation (58%) – generally associated with mild anaemia and marginally higher IgM, followed by B-symptoms (33%), peripheral neuropathy (29%), leg cramps (17%) and epistaxis (11%). Median IgM at diagnosis was 26.5g/L (0.5–79g/L) and haemoglobin 117g/L (44-157g/L), whereas at first treatment, medians were 30.6g/L (0.5-79g/L) and 101g/L (44-154g/L), respectively. 24 different first-line therapeutic combinations were used in 85 participants.

Conclusion

This update demonstrates a robust data-collection platform via CART-WHEEL.org and the feasibility of its use globally for WM patient-derived data. Upon further recruitment, the information gathered will expand knowledge of the range of presentations and treatment experiences of WM patients. Real-world therapeutic efficacy will be highlighted, along with international disparities of treatment access.

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197. Primary Extranodal Marginal Zone Lymphoma of the bladder and its outcomes

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Aim

Primary Marginal Zone Lymphoma of the bladder is a rare presentation, with fewer than 15 cases described in the literature. We review a case complicated by hydronephrosis, and its initial treatment.

Case Presentation

The patient presented following three months of intermittent haematuria and sterile pyuria, on a background previous bowel cancer, and bronchiectasis. Initial imaging of her abdomen revealed bladder wall thickening, as well as right sided ureteric thickening and hydronephrosis. No other lymphadenopathy was demonstrated. Cystoscopic biopsy revealed atypical lymphoid cells of small to intermediate size with some showing monocytoid and plasmacytoid features. Immunohistochemistry showed an atypical lymphoid infiltrate to be CD20+, and negative for T cell markers, Cyclin D1 and CD10. No reciprocal t(11;18) translocation was demonstrated by FISH. The diagnosis of stage 1AE extranodal marginal zone lymphoma of mucosa associated tissue was made. She was treated with radiotherapy with curative intent. She received 30 Gy over 20 fractions, and responded well to therapy. She is now waiting restaging and further follow up.

Discussion

Lymphoma of the bladder can either be defined as primary or secondary. Primary extranodal MZL is a rare entity, with only 10 cases reviewed in the literature. The patient had a classic presentation, defined by intermittent haematuria, and urinary frequency. Unlike other MALT lymphoma, her case was not preceded by recurrent urinary tract infections and antigen exposure, which may alter the current pathophysiology of the disease. The biopsy sample was also classic for MZL, and there was no evidence of pathognomonic cytogenetic abnormalities. Finally, treatment with curative radiotherapy proved to be effective, which may provide evidence to avoid chemotherapy and still attain excellent clinical outcomes

Conclusion

Primary MZL of the bladder is a rare entity, and its optimal treatment is still unknown. Here we present a case with obstructive uropathy that was treated successfully with radiation. Further randomized controlled trials are still needed regarding this disease.

There is no conflict of interest

198. False negative hepatitis C serology following rituximab-based chemotherapy: a case report

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Introduction

Rituximab-based chemotherapy is the standard treatment in CD-20 positive non-Hodgkin lymphoma. While rituximab is known to reduce seroconversion and antibody responses to influenza and pneumococcal vaccinations,^{1,2} false negative serology in hepatitis C virus (HCV) has not been reported in the literature. We report a case of HCV infection with false negative HCV serology following rituximab-based chemotherapy.

Case report

A 38-year-old gentleman with Stage IVBES relapsed transformed follicular lymphoma was treated with 2 cycles of salvage chemotherapy R-ICE with view of autologous stem cell transplant (ASCT). His previous treatment included 6-cycles of R-CHOPq21 followed by 6-months of rituximab maintenance before relapse. During routine viral surveillance following stem cell collection, nucleic acid testing for hepatitis C was found to be positive. Hepatitis C serology were repeatedly negative including at baseline prior to chemotherapy in 2015. A repeat serum HCV RNA 4 weeks later showed high viral load but again negative anti-HCV antibodies as well as other viral serologies including HBV and HIV.

The timing of HCV acquisition is unable to be determined. It is postulated that B cell dysfunction related to rituximab and intensive chemotherapy has resulted in lack of immune response against recently acquired HCV infection. He is an ex-intravenous drug user although did not report recent use. There were no other risk factors for HCV infection apart from blood transfusion without reported transfusion-transmitted infection. Treatment and reassessment of HCV were deferred to post ASCT to detect antibody response upon immune recovery.

Discussion

To our knowledge this is the first case of false negative HCV serology following rituximab-based chemotherapy. It raises the question of whether the use of routine nucleic acid testing is indicated in immunosuppressed patients who are at high risk of acquiring blood-borne virus infection.

Conflict of Interest

The authors declare no conflict of interest.

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199. High-dose methotrexate with high-flux haemodialysis in treatment of post-transplant lymphoproliferative disorder with ESKD

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Background

High-dose intravenous methotrexate (HD-MTX) is an integral part in primary central nervous system (CNS) lymphoma treatment. Predominant renal excretion of methotrexate limits its use in patients with end-stage kidney disease (ESKD). We report a case of post-transplant CNS lymphoma treated with methotrexate and extended hours high-flux haemodialysis.

Case Report

A 41-year-old man with a cadaveric renal transplant of 26 years for reflux nephropathy, presented with headache and absence seizures despite previously well-controlled epilepsy. He had chronic allograft nephropathy on triple therapy immunosuppression. Multi-focal lesions found on cerebral magnetic resonance imaging (MRI) were biopsied to confirm monomorphic post-transplant lymphoproliferative disorder (PTLD) without other sites of disease. Transition to haemodialysis was complicated by increased clearance of anti-epileptic drugs (AEDs) leading to partial seizures. Whole brain radiotherapy (WBRT) was undertaken for initial control, followed by three cycles of dose-escalated methotrexate. This was followed by eight-hour high cut-off haemodialysis, and leucovorin rescue with close monitoring of methotrexate levels and increased dose of AEDs. Follow-up MRI, and neurological PET-CT confirmed remission 6 weeks' post-treatment.

Methotrexate is primarily cleared in the urine, which limits its use in ESKD due to nephrotoxicity and myelosuppression. In conjunction with high-flux haemodialysis, HD-MTX ranging from 1 to 9.5g/m² has been scarcely described for solid organ and haematological malignancies. Confounding management issues included the associated clearance of AEDs with extended-hours haemodialysis. This was managed with intra-dialytic drug dosing and up-titration. Adequate clearance of methotrexate occurred despite increased dosing. Significant residual renal function may have contributed to prompt drug clearance.

Conclusions

For CNS lymphoma, HD-MTX with high-flux haemodialysis should be considered a viable option in candidates with ESKD without other contraindications.

200. Concurrent marginal zone lymphoma and tuberculosis: a case report

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Case

A 70 year old woman presented with a progressively enlarging right neck mass which had been present for 15 years. She was otherwise asymptomatic and had immigrated to Australia 42 years prior from Greece. Examination revealed no other lymphadenopathy or organomegaly. Histology on excisional biopsy demonstrated non-necrotising granulomatous inflammation. Ziehl-Neelsen stain demonstrated acid fast bacilli and the culture which was positive for mycobacterium tuberculosis.

Flow cytometry and immunohistochemistry of the lymph node demonstrated a monoclonal B cell population, CD20+, CD3-, CD5-, CD10-, bcl-2+, CD23- in keeping with concurrent nodal marginal zone lymphoma.

She was started on rifampicin, isoniazid, pyrazinamide, ethambutol and pyridoxine for two months before de-escalation to rifampicin and isoniazid, completing a six-month course. Her stage IIIAS marginal zone lymphoma was observed until she developed widespread rash 3 years post her initial diagnosis. The rash was confluent and erythematous over her face with nodules on her chin, discoid dermal papules and plaques and nodules over her chest, upper arms, abdomen and darker violaceous plaques on her buttocks.

Biopsy revealed atypical cells which were positive for CD20 and negative for CD5, CD10, CD23 and cyclin D1. Bone marrow biopsy was undertaken revealing involvement. Rituximab, cyclophosphamide, vincristine and prednisolone chemotherapy was favoured over bendamustine and rituximab due to the lower degree of immunosuppression given her history of mycobacterial lymphadenitis. Her rash markedly improved and after six cycles she achieved a complete remission radiologically. She continues to be in complete remission to date.

Conclusion

Non-Hodgkin's lymphoma and tuberculosis co-existence in the same location is rare and described only in limited case reports. This case highlights the importance of considering concurrent entities prior to undertaking treatment for either condition.

No conflict of interest to disclose

201. Apoptotic activity in Myelodysplastic Syndrome: relation to peripheral blood counts and dysplastic features

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Aim

To investigate the involvement of apoptosis in the pathogenesis of Myelodysplastic Syndrome (MDS).

Method

Ten bone marrow aspiration sample were collected from patients with peripheral cytopenias from Hospital Universiti Kebangsaan Malaysia and Hospital Ampang, Malaysia. The bone marrow aspiration smear were analysed to detect MDS cases. The expression of the death receptor, Fas (CD95/Apo-1), was analysed on specific cell markers and the apoptotic index (AI) was determined by Annexin V binding assay and TUNEL assay on bone marrow cells. The association and correlation between expression of apoptotic markers with peripheral blood counts and dysplastic features of bone marrow were determined using IBM SPSS Statistics ver 20.0. T-test (parametric sample) and Mann Whitney test (non-parametric sample) were used to analyse the data while Pearson's (parametric sample) or Spearman's test (non-parametric sample) were used for correlation analyses.

Results

Seven out of 10 cytopenia cases were morphologically suggestive as MDS. Results showed that the expression of Fas (CD95/Apo-1) on CD235a+ was significantly elevated in suggestive MDS cases as compared to controls. The expression of this death receptor was also slightly elevated on CD33+, CD34+ and CD45+ of bone marrow. However, this was not statistically significant. There is no significant difference in the apoptotic index between MDS cases and controls. Nevertheless, trends showed AI was higher in MDS than controls. A significant but inverse relationship was observed between Fas (CD95/Apo-1) expression on granulocytes markers and AI. The expression of Fas (CD95/Apo-1) on others compartment of bone marrow cells had no correlation with AI. The AI have no correlation with peripheral cytopenia. Furthermore, the dysplastic features identified in bone marrow aspiration smears showed no correlation and association with AI.

Conclusion

Fas (CD95/APO-1) may not be the only mediators to initiate apoptosis in MDS. The lack of correlation between the apoptotic activity with reduction of peripheral blood counts and dysplastic features of bone marrow may suggest that cytopenias in MDS are multifactorial.

202. An unusual case of del(5q) and t(9;22) in a patient with myelodysplastic syndrome

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Aim & Method

We present a case of an 80-year-old woman with transfusion-dependent pancytopenia who was diagnosed with type 2 refractory anaemia with excess blasts. Interestingly, cytogenetics performed on the bone marrow showed 17/20 metaphases carried del(5q) and 3/20 carried t(9;22). Investigation of t(9;22) using RT-PCR detected m-BCR which is associated with Ph-positive ALL. While the literature describes a few cases of combined del(5q) and t(9;22), this picture has not been described previously.

Result

The patient was initially approved to receive azacytidine, but following cytogenetics, she was changed to lenolidamide which is well established in treating del(5q). While tyrosine kinase inhibitors (TKIs) are shown to be effective in treating t(9;22), the use of TKIs has been deferred due to the patient's age and risk of excessive bone marrow suppression.

For several months, the patient has been transfusion dependent for anaemia. She has borderline thrombocytopenia and although leucopaenic, her white cell count has been stable over the last five months. Currently BCR-ABL1 translocation levels are low at 2.44%, but careful monitoring with qPCR bcr.abl is being maintained with the view to introduce a TKI or other therapy if required.

Conclusion

This case reports an unusual genetic combination in myelodysplastic syndrome (MDS) and highlights the value of cytogenetics in tailoring treatment. As a sole abnormality, del(5q) is generally associated with favourable prognosis. In contrast, t(9;22) although characteristic of CML, is also observed in ALL - an aggressive disorder with poor prognosis in adults. The prognosis of this combination is unknown, however, knowledge of the cytogenetics offers the possibility of targeting treatment with lenolidamide and TKIs. This case also illustrates the challenges in managing MDS in an elderly patient when a sequential approach to immunotherapy is required and options such as allograft are unavailable.

203. Role of flow cytometry in diagnosis of myelodysplastic syndrome

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Aim

Myelodysplastic syndromes (MDS) present with diverse and subtle immunophenotypes and are thus difficult to profile by flow cytometry. A 4-point scoring system (the Ogata score) has been proposed as an objective method to evaluate flow cytometry data for MDS (Della Porta MG et al 2012, *Haematologica*, 97:1209-1217). This study aims to improve MDS diagnosis by retrospectively applying the 4-point score to flow cytometry data obtained from bone marrow samples taken for assessment of possible MDS.

Method

The study was carried out on bone marrow aspirate samples taken at a major teaching hospital in Sydney, for evaluation of possible or known MDS or MDS/MPN. Samples with other haematological malignancies were excluded. Flow cytometry data were analysed in Kaluza® software according to the 4-point score using forward- and side-scatter, CD19, CD33, CD34 and CD45. The 4 elements, myeloblast-related cluster size, B-progenitor-related cluster size, lymphocyte-to-myeloblast CD45 ratio and granulocyte-to-lymphocyte side-scatter ratio, were determined, and samples with 2 or more criteria outside the reference range were considered to be MDS by flow cytometry. The data were assessed in terms of diagnostic sensitivity and specificity with comparison to bone marrow aspirate and trephine cytomorphology, and cytogenetics.

Result

It has been straightforward to apply the 4-point system to stored flow cytometry data. To date, 92 samples have been analysed, and further samples are being assessed. The data on the initial 92 samples indicate the 4-point system sensitivity is 77% and specificity is 90.5% in separating MDS samples from non-MDS samples.

Conclusion

We predict that this study will show the objective 4-point score to be a useful adjunct to conventional cytomorphology and cytogenetics.

206. JAK1 somatic mutation in myeloproliferative neoplasm

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Aim

For most triple-negative myeloproliferative neoplasms a molecular diagnosis is not available because access to clinical next generation sequencing (NGS) is limited or expensive. In addition, there are cases which are difficult to fit into the WHO criteria for MPNs. We employed a custom designed NGS panel to identify potential driver mutations in a case of triple-negative MPN.

Methods

Routine review of the blood peripheral morphology and bone marrow histology of our patient (a 78-year-old woman presenting with leucocytosis, left shift and thrombocytopenia) was undertaken. A provisional diagnosis of MPN-U was made. Testing for BCR-ABL, JAK2, CALR and MPL mutations was negative. A comprehensive NGS panel was therefore employed to search for a responsible driver mutation (Magor, JMD, 2016).

Results

Two single nucleotide variants (SNVs) were detected in >200kb of sequenced DNA: (i) a heterozygous SNV in JAK1 leading to a predicted missense mutation, V658I; and (ii) a SNV in ETV6 which was considered unlikely to be pathogenic. The V658 amino acid in JAK1 is equivalent to V617 in JAK2, and transduction of JAK1-V658I leads to factor independent cell growth of BaF3 cells, so it is likely pathogenic. The DNA sequences of the exons of JAK2, MPL, CALR, CBL, SETBP1, SRSF1 and 86 other genes on the panel, including those previously linked with aCML and CMML, were normal.

Conclusions

To our knowledge, JAK1 mutations have not been described in MPNs. We suggest consideration of JAK1 mutations should be undertaken in cases of MPN-U, aCML or CMML which do not have a molecular diagnosis. Since these diseases are heterogeneous, a broad NGS panel is most likely to yield the responsible driver mutation. This genetic information is of clinical value since the disease is likely to respond to JAK inhibitors which have affinity for JAK1, such as ruxolitinib.

207. Evaluation of the QIAGEN® CALR RGQ PCR for the detection calreticulin mutations in essential thrombocytosis

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Frame shift mutations in exon 9 of the Calreticulin(CALR) gene are known to be driving mutations of essential thrombocytosis(ET) and primary myelofibrosis(PMF). Approximately 60% of CALR mutations are recurrent 52bp deletions(type 1), 40% are 5bp insertions(type 2), whilst the remaining 10% are rare insertions or deletions. Type 1 mutations have been associated with increased progression of ET to PMF and improved overall survival, whilst type 2 mutations have been associated with higher platelet counts in ET, higher blast percentages in PMF and poorer overall survival.

Diagnostic testing for CALR mutations predominantly utilise PCR and capillary electrophoresis fragment length analysis(CE) to identify possible insertions or deletions. Whilst this method is effective at identifying the presence of a possible frameshift mutation, time consuming sequence analysis is required to truly characterise the mutation and confirm the loss of the critical KDEL endoplasmic reticulum retention motif.

The CALR RGQ PCR Kit by QIAGEN® provides an alternative method to directly identify type 1 and type 2 CALR mutations whilst also screening for rare non-type 1 and type 2 mutations. The methodology uses 2 specific ARMS PCR for the identification of type 1 and type 2 mutations and 5 CLAMP inhibited PCRs for detection of other exon 9 mutations. A comparative study(n=31) was made between the RGQ method and PCR/CE with confirmation by Sanger sequencing.

The RGQ method was found to be 100% concordant with the capillary electrophoresis method for type 1(9/9), type 2 mutations(9/9) and wild type (4/4), however detected only 44% of the non-type 1 or type 2 mutations(4/9). Overall concordance with the reference method was calculated at 84%.

These results suggest the kit is effective in directly identifying type 1 and type 2 CALR mutations and eliminating the need for confirmatory sequencing, however rarer mutations are likely to be missed using this method.

208. Immature platelet fraction may be used to predict JAK2 V617F mutation-positive polycythaemia vera patients

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Aim: In 2016 the WHO lowered its haemoglobin (Hb) thresholds for diagnosing polycythaemia vera (PV) to 165 g/L for men or 160 g/L for women.¹ This has considerable resource implications for laboratories considering whom to screen for JAK2 mutations. JAK2 V617F mutation is associated with elevated levels of immature platelet fraction (IPF) an inexpensive parameter measured by Sysmex XN-series analysers.² We aimed to establish a reference range for IPF and to evaluate a simple algorithm based on complete blood count (CBC) indices, including reduced mean cell volume and increased platelets, neutrophils or basophils, designed to identify potential PV patients, including those presenting with normal or mildly elevated Hb levels.

Method: An IPF reference range was derived from 102 patients having routine screening blood counts with no abnormal indices or flags.

A total of 20,009 CBC were screened for potential PV by our proposed algorithm, identifying 39 potential patients for IPF testing. IPF testing was also performed on patients with known JAK2 mutation status.

Results: A frequency distribution curve was plotted for the reference range subjects. A Gaussian distribution was identified and a 2 SD reference range was derived.

Four groups were identified in addition to the reference population: 1) JAK2 mutation-positive patients not receiving hydroxycarbamide; 2) JAK2 mutation-positive patients on hydroxycarbamide therapy; 3) patients with raised IPF with JAK2 status not determined; and 4) patients with normal IPF and negative JAK2 testing.

Box and whisker plots were compiled for the four study groups which demonstrate good separation of IPF values between the groups (figure 1).

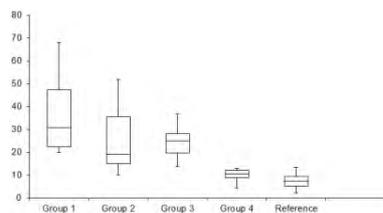


Figure 1

Conclusion: By implementing an algorithm in our middleware we are able to identify a group of patients who have either normal or only mildly elevated indices who are likely to be JAK2 mutation-positive, markedly reducing the number of JAK2 mutation screening tests required.

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209: Ruxolitinib: The experience of a single centre in Singapore

Kam G¹, Lim Y¹, Ng H¹, Chuah C¹, Tan C¹, Ang A¹, Grant D¹, Lee Y¹, Tay H¹, Teo E¹, Wong G¹, Yiu R¹, Hwang W¹, Goh Y¹, Lee L¹

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Aim

Myelofibrosis is a myeloproliferative neoplasm (MPN) characterized by splenomegaly, debilitating constitutional symptoms and poor quality of life. It may arise de novo as primary myelofibrosis, or progress from prior essential thrombocythaemia or polycythaemia vera. The oral Janus kinase (JAK1/ JAK2) inhibitor ruxolitinib has been demonstrated to effectively improve splenomegaly and myelofibrosis-related constitutional symptoms. Prior to this, there was no therapy to effectively ameliorate these symptoms. This analysis reviews the efficacy and tolerability of ruxolitinib in patients with MPNs treated at our institution.

Method

We retrospectively reviewed the clinical records of 25 patients from Singapore General Hospital, Singapore with MPNs treated with ruxolitinib for at least one month. Patients were started treatment from September 2011 to December 2015. The decision to start ruxolitinib was based on the discretion of the managing physician. Doses were adjusted to optimize efficacy and minimize toxicity.

Result

Twenty-three patients had myelofibrosis (60.8% intermediate-2/ high risk), 1 had polycythemia vera and 1 had myeloproliferative neoplasm-unclassifiable. Duration of follow up was 25.8 months (1.6-62.3 months). 56% were male and 80% JAK2 V617F positive. The median time to commencing ruxolitinib from diagnosis of myelofibrosis was 23.1 months (0.7-260.6). 88% had received prior therapies for their MPN. 80% had splenomegaly and 70% had a spleen > 10cm below the left costal margin. 36% had hepatomegaly and 72% had constitutional symptoms. Although the median starting dose was 40mg/day, over 23.3 months of treatment, 52% required a dose reduction. At last review, median dose was 20mg/day. All had an improvement in constitutional symptoms, 40% had a reduction in splenomegaly, and 88.9% a decrease in hepatomegaly. Improvements in hepatomegaly and splenomegaly occurred within a month and were maintained for 21 months and 16 months, respectively. No significant toxicities were observed.

Conclusion

Ruxolitinib was well tolerated and produced rapid sustained improvements in splenomegaly, hepatomegaly and constitutional symptoms.

No conflict of interest to disclose

210. Clinical relevance of quantitative JAK2V617F in Polycythaemia Vera and Essential Thrombocythaemia in a local cohort.

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Aim

Patients with polycythaemia vera (PV) have a higher JAK2V617F allelic burden compared to essential thrombocythaemia (ET) (Rumi E, 2014; Vainchenker W, 2017); however clinical relevance of this is unclear. Quantitative JAK2V617F analysis is not routine practice at Christchurch Hospital. The aim of this study was to determine the JAK2V617F allelic burden at diagnosis in a local cohort of patients with PV and ET and correlate the results with traditional risk factors for morbidity and mortality.

Method

Lists of 40 consecutive patients above the age of 18 with JAK2V617F positive PV and JAK2V617F positive ET, diagnosed between 2005 and 2010, were generated from a local database. All patients had stored DNA obtained from peripheral blood leucocytes at diagnosis. The JAK2V617F allelic burden of each diagnostic sample was determined using the Bio-Rad droplet digital PCR Qx200 analyser.

The following retrospective variables were obtained from clinical records – age at diagnosis, white cell count at diagnosis, vascular events, transformation to myelofibrosis or acute leukaemia and mortality.

Results

There was no significant difference in age or gender distribution between the PV and ET cohorts. There was a statistically significant difference between the mean JAK2 V617F allelic burden between the PV group (mean burden – 50.79%) and ET group (mean – burden 23.51%) ($p < 0.0001$). No patients with ET had an allelic burden of more than 50%. There was no significant correlation between the other clinical variables analysed and the JAK2V617F allelic burden in either group.

Conclusion

The statistically higher JAK2V617F allelic burden in our PV patients compared to ET patients is consistent with the literature. Given the small sample size, larger, prospective studies are required to further evaluate the relationship between allelic burden and the traditional clinical risk factors and disease specific patient outcomes in JAK2V617F positive MPNs.

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VAINCHENKER, W. KRALOVICS, R et al. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. **Blood**, v. 129, n. 6, p. 667 - 679, 2017.

211. An Interesting Case of Essential Thrombocythaemia Masquerading as a Transformed Disease

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Introduction: Patients with the MPN Essential thrombocythaemia (ET) or Polycythaemia vera (PV) are susceptible to transformation to myelofibrosis¹, which may be suggested by the development of pancytopenia or a leucoerythroblastic film. We present a patient with stable ET on hydroxyurea (HU) who presented with pancytopenia and a leucoerythroblastic blood film, to illustrate the importance of a broad differential when approaching this problem.

Method/Case History: A 59-year-old patient with JAK2 V617 positive ET in 2014 on a variable dose of HU in addition to Aspirin to control the thrombocytosis due to vascular risk factors including tobacco use. He presented in March 2017 during routine follow up with clinical features of symptomatic anaemia and mild splenomegaly. A bone marrow aspirate (dry tap) and trephine biopsy showed variable cellularity, reduced trilineage haematopoiesis and extensive fibrosis surrounding an extensive interstitial infiltrate of non-haematopoietic cell colonies. These cells contained melanin and were confirmed on immunostains as melanoma. (S100 and SOX10). A primary skin lesion was biopsied and was consistent with melanoma, with no evidence of BRAF, NRAS and KIT mutation evident on next generation sequencing. Staging CT and PET showed widespread metastatic melanoma suspected to have arisen from a primary lesion on the back. The patient's HU was ceased and he received a single dose of pembrolizumab, which was complicated by drug-induced hepatitis, which proved to be fatal.

Discussion/Result: Post-ET–MF complicates about 0.8 - 4.9% of ET¹, often accompanied by progressive cytopenias, splenomegaly and constitutional symptoms. The most common cytoreductive therapy for ET is HU, which has been associated with development of non-melanomatous skin cancers² however there have been no reports of HU associated malignant melanoma. Rather there are some case reports indicating successful therapy of malignant melanoma.³

Conclusion: A leucoerythroblastic blood film and fibrotic marrow is not synonymous with transformation to myelofibrosis, and as such, marrow involvement with secondary cancers should also be considered. Clinicians should aim to use the lowest dose of HU possible to maintain an acceptable platelet count and minimise secondary malignancy risk.

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1994Volume 30, Issue 7,1027–1029

Figures: Fig 1: Bone marrow trephine biopsy : H&E and Reticulin Stain (40X)



213. Classifying monocytes and its precursors: a study on concordance in morphology

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Aim

To examine the concordance in classifying monocytes and monocytic precursors amongst laboratory scientists, haematology trainees and haematologists in Australia.

Rationale

Monocytes and its precursors remain one of the most difficult cells to classify morphologically¹. According to WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues 2008, classification into monoblasts, promonocytes, abnormal monocytes and mature monocytes can be made based upon presence of nucleoli, nuclear chromatin pattern, nuclear indentation and cytoplasm characteristics². Whilst immunophenotyping based on CD33, CD34, CD14 & CD45 expression has been used to distinguish between monoblasts, promonocytes and mature monocytes³, morphology remains essential in several diagnoses, including acute myelomonocytic leukaemia (AMML), acute monoblastic and monocytic leukaemia as well as chronic myelomonocytic leukaemia (CMML). Goasguen et al published a high concordance in sub-classification of monocytes using proposed criteria¹. We would like to examine the concordance in monocyte morphology reporting in the Australian setting.

Methods

Digital images of 20 monocytic-lineage cells from patients with leukaemic proliferation of monocytes will be included in this poster. We will conduct a pilot study examining concordance in reporting amongst laboratory scientists and haematologists at our institution, the results of which will be included in this poster. Attendees at the HSNZ scientific meeting will be encouraged to participate in a blinded survey to classify these images into 4 subclasses: monoblasts, promonocytes, immature monocytes and mature monocytes. The results will be analysed according to responders' role and experience (number of years / stream of specialty) in morphology.

Results

Our pilot study results will be included in this poster. Survey results from HSNZ attendees will be analysed and reported at RCPA Pathology Update in March 2018.

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214. Melphalan dose in autologous stem cell return for multiple myeloma

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Aim

To review clinical outcomes of stem cell return (SCR) in multiple myeloma patients based on Melphalan dose.

Method

A retrospective observational study of 1st SCR who had bortezomib-based chemotherapy in a single centre. Patients either received 200mg/m² of melphalan (Mel200) or 140mg/m² of melphalan (Mel140). Primary outcomes were length of hospital stay, time for engraftment, duration of intravenous(IV) antibiotics. Secondary outcome was time to death or relapse in the first 12 months.

Results

43 patients were eligible. There were 33 patients in Mel200 group and 10 in Mel140 group. The mean age (\pm standard deviation) of patients was 55.8(\pm 7.8) years in Mel200 group while it was 66.9(\pm 1.9) years in Mel140 group ($p < 0.001$). Renal function, stage of myeloma, induction regime and disease status at transplant were similar in both groups. The average inpatient days (\pm standard deviation) and days on IV antibiotics were 19.76 (\pm 9.8) and 8.5 (\pm 6.9) respectively in Mel200 group while they were 22.3(\pm 9) and 8(\pm 5.3) in Mel140 group ($p > 0.05$). The average days for neutrophils and platelets engraftment were 17.7(\pm 7.8) and 29.8(\pm 27.8) respectively in Mel200 group and were 17.6(\pm 4.9) and 28.6(\pm 10.4) in Mel140 group ($p > 0.05$). In terms of event-free survival, there were 4 events in Mel200 and 2 events in Mel140 group at 12 months ($p > 0.05$).

Conclusion

The dose of melphalan has no impact on hospital stay, duration of antibiotics, days until engraftment and survival. However, elderly patients in Mel140 group were probably highly selected.

215. Comparison of immunohistochemistry and multi parameter flow cytometry for the characterisation of malignant plasma cells.

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Aim

Assessing the immunophenotype of plasma cells is central to the characterisation of plasma cell dyscrasias at diagnosis. Demonstrating plasma cell aberrancy requires either immunohistochemistry (IHC) or multi parameter flow cytometry (MPFC). Herein we aim to review the performance of and concordance between these two methods for the detection of aberrant plasma cells.

Methods

Fifty one patients with multiple myeloma were identified from institutional databases at the Royal Melbourne Hospital. Bone marrow samples acquired at diagnosis were assessed by IHC (bone marrow trephine) and MPFC (bone marrow aspirate). IHC was performed using immunostains for CD20, CD56 and CD117. MPFC was performed using the Beckman Coulter Navios flow cytometer using the following fluorochrome combination: CD45, CD38, CD138, CD19, CD56, CD20, CD117, CD27, CD28 and CD81. MPFC data was analysed using Kaluza Analysis Software 1.5a (Beckman Coulter) and aberrancy defined by expression of CD56, CD28, CD117, CD20 or loss of CD19 or CD27 expression. Both IHC and MPFC data were interpreted independently by two haematopathologists.

Results

100% (51/51) of cases displayed plasma cell aberrancy by MPFC. Of the 18 samples positive for CD117, discordance between methodologies occurred in 22% (11/18) of cases, the vast majority (91%, 10/11) being positive by IHC alone. Thirty five samples were positive for CD56, of these 26% (9/35) displayed discordance, 22% (2/9) being positive by IHC alone and 78% (7/9) by MPFC alone. CD20 expression was detected in 39% (20/51) samples, six of which (30%) were evenly discordant. In total 31% (16/51) of cases displayed discordance between IHC and MPFC. IHC appeared to perform better for CD117 detection whilst CD56 detection appeared to be favourable by MPFC.

Conclusion

Discordance between IHC and MPFC was demonstrated at 31% and appears to be antigen dependent. It is recommended that these two methods be considered complementary, particularly in cases where bone marrow aspirate quality may result in suboptimal MPFC performance or in cases where plasma cell aberrancy is not initially apparent by MPFC.

216. DiCAM (DiChloroAcetate in Myeloma): Interim trial results suggest revision of the recommended DCA dose regimens

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Background

Malignant cells display distinct metabolic phenotypes, including the glycolytic phenotype (prominent glycolysis even in the presence of adequate oxygen). Some multiple myeloma (MM) demonstrates this phenotype. Dichloroacetate (DCA) is an investigational drug that reverses the glycolytic phenotype by inhibiting pyruvate dehydrogenase kinases (PDK), redirecting metabolism of pyruvate away from lactate production towards mitochondrial oxidation. Several phase 1/2 trials in solid cancers suggest DCA shows promise as synergistic with chemotherapy.

Methods

DiCAM, a phase 2 study at The Canberra Hospital, aims to determine efficacy of DCA in 'plateau phase MM' as indicated by a >25% fall in paraprotein and/or free light chain difference. DCA was administered as a novel loading dose of 25mg/kg BD for 5 doses, followed by a standard 6.25mg/kg BD for 3 months, based on previously reported dosing regimens. Peak and trough levels were correlated with tolerability and *GSTZ1* genotype, the liver enzyme responsible for DCA's metabolism.

Results

To date, six patients have completed treatment. Three showed a partial response after the first month; not sustained at 2 months. Two maintained a stable plateau, one patient progressed. Trough DCA levels on maintenance dosing (0.1-0.15 mM) approached the level for on-target inhibition of constitutive PDK2 (0.2 mM), but not hypoxia-induced PDK1 and PDK3. A 25mg/kg dose resulted in sustained (>6 hr) levels of 0.4-0.6 mM.

Minor sensory neuropathy was the most common adverse effect. One patient with prominent neuropathy showed higher DCA troughs, and possessed an uncommon *GSTZ1* promoter genotype (11%) associated with reduced promoter activity.

Conclusion

DCA shows modest efficacy with low toxicity making it suitable for combination myeloma therapy. Dosing regimens used in prior trials may not be adequate to inhibit PDK, particularly with varying *GSTZ1* promoter genotypes. Further trials with higher dose regimens and expanded inclusion criteria encompassing patients with smouldering myeloma are planned.

217. Polynesian multiple myeloma: a single centre study from the ANZ Myeloma and Related Diseases Registry

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Aim

To compare characteristics and outcomes of Polynesian (NZ Maori and Pacific Island) patients with others with multiple myeloma (MM) in a New Zealand (NZ) hospital.

Methods

Data for all patients with MM from Middlemore Hospital in South Auckland, NZ, registered on the Myeloma and Related Diseases Registry (MRDR) from Jan 2013 - Jun 2017, were analysed. Polynesians were those who self-identified as NZ Maori or Pacific Islander and had at least one Polynesian grandparent. The comparator group included patients of other ethnicities, predominantly European.

Results

From a population of 525,000 (15.7% NZ Maori, 21.5% Pacific Islanders and 62.8% other), data on 139 MM patients were available; 56 (40%) were Polynesian (includes NZ Maori and Pacific Islanders) and 83 (60%) of other ethnicities. Polynesians were younger at diagnosis (median age 63y v 70y, $p < 0.001$) and less were >70 years (29% v 52%, $p < 0.001$). The sex distribution was similar in both groups (males 41% v 53%, $p = 0.17$), as was the proportion with high risk features based on FISH and LDH (32 v 36% high risk, $p = 0.63$); however Polynesians had worse ECOG at presentation (ECOG 2-4, 27% v 10%, $p = 0.02$). The proportion of Polynesians with diabetes, bone lesions and renal impairment at diagnosis were similar (all $p > 0.05$).

A similar proportion of Polynesian patients received a bortezomib/ cyclophosphamide/ dexamethasone regimen (89% v 95%); the response rate was 79% in Polynesians and 93% in others ($p = 0.057$). Also the autologous transplant rate (47% v 71%, $p = 0.13$) was not statistically different. Median progression-free survival was similar (32.1 v 29.5 mths, ($p = 0.55$), but there was a trend to reduced overall survival (33.8 v 43.5 mths, $p = 0.049$), respectively.

Conclusions

Polynesian patients are diagnosed at a younger age with similar disease characteristics but worse performance status. Attention is drawn to the reduced survival in the Polynesian group, which is a continuing focus for practice improvement.

218. Cytogenetic analysis retains prognostic significance in multiple myeloma patients treated with novel therapies

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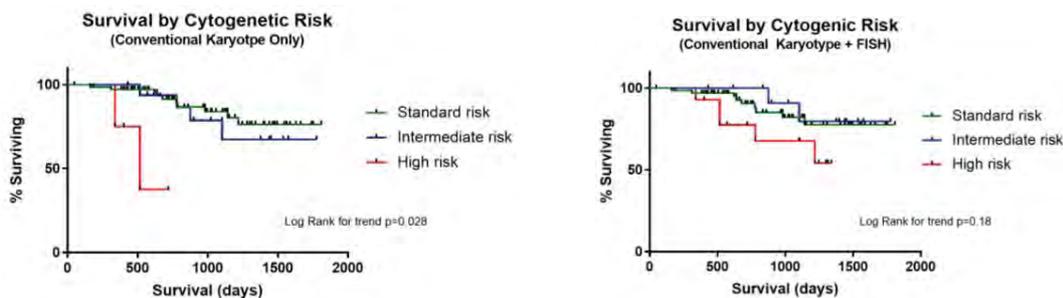
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Aim: Though previously considered a fundamental prognostic investigation, the role of conventional karyotyping for multiple myeloma in the era of novel therapies has been questioned. Some groups advocate relying solely on FISH studies to identify major cytogenetic abnormalities.¹ We sought to assess the prognostic significance of cytogenetic abnormalities in patients who have received novel therapies including bortezomib, thalidomide, lenalidomide carfilzomib and pomalidomide, focusing on the role of conventional karyotyping.

Methods: We retrospectively analysed 107 patients with multiple myeloma, treated at the Alfred Hospital between February 2013 and December 2015. Baseline karyotype and FISH analyses were reviewed where available. Survival analysis was performed via the Kaplan Meier method, and survival between groups was assessed via the Log Rank test.

Results: The median age at diagnosis was 61 years (range 21-86), 65% were male. Median duration of follow-up was 28 months. Conventional karyotyping was performed for 90 patients (84%) and FISH studies were performed for 83 patients (78%). All patients were exposed to a novel therapy, and 104 (97%) received bortezomib. In the entire cohort the international staging system (ISS) and revised ISS (R-ISS) score showed a trend when correlated with overall survival, though this was not statistically significant ($p=0.058$ and $p=0.15$ respectively). When allograft recipients were excluded, similar results were obtained. Cytogenetic risk stratification was performed according to IMWG consensus guidelines.² Whilst risk stratification utilising combined conventional karyotyping and FISH data did not significantly predict overall survival, stratification utilising conventional cytogenetics alone was significantly associated with survival in the entire cohort and when allograft recipients were excluded ($p=0.0009$ and $p=0.0379$ respectively, Fig 1&2)

Discussion: Though limited by small numbers, our data demonstrates the continuing prognostic relevance of conventional karyotyping in patients treated with novel therapies, and supports an ongoing role for cytogenetic analysis in addition to FISH studies in the management of multiple myeloma.



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219. Socio-Economic Deprivation is Associated with Inferior Survival in Patients with Newly Diagnosed Multiple Myeloma

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Aim: The aim of this is to evaluate the impact of socio-economic (SE) deprivation on overall survival (OS) amongst patients with newly diagnosed multiple myeloma (MM) in New Zealand, and assess whether the introduction of bortezomib has changed the its impact.

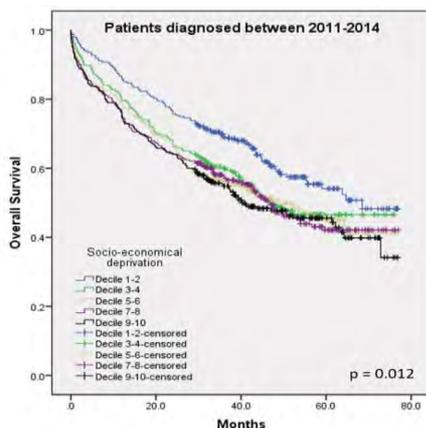
Method: Bortezomib was funded in New Zealand (NZ) from May 2011. Cases diagnosed between 2011-2014 (*bortezomib-era*) were compared against those diagnosed in the preceding 4 years (*pre-bortezomib-era*). Data were extracted from the New Zealand national cancer registry and linked to the 2013 New Zealand Index of Deprivation by the patients' domicile code. The degree of SE deprivation was divided into deciles with 1 being the least deprived and 10 being the most deprived. All statistical analyses were performed using SPSS version 20.

Result: A total of 2425 cases were analysed with 1076 diagnosed *pre-bortezomib* and 1349 in *bortezomib-era*. In the *bortezomib-era* there was a greater proportion of male patients compared with *pre-bortezomib-era* (59.9% vs 55.2%, $p = 0.032$). The median age was similar between the two time-periods ($p = 0.866$). SE deprivation was not a significant factor for overall survival (OS) in the *pre-bortezomib-era* ($p = 0.153$), but was significant in *bortezomib-era* ($p = 0.012$). SE remained as an independent factor in the *bortezomib-era* on multivariable analysis (HR 1.074, $p = 0.015$). Patients in all SE deprivation deciles experienced an improvement in overall survival in the *bortezomib-era* comparing to *pre-bortezomib-era*, but those who were least deprived (decile 1-2) experienced the greatest margin of improvement (table 1).

Conclusion: Following the introduction of bortezomib, OS for patients with MM has improved across the board regardless of the degree of SE deprivation. However, for patients diagnosed between 2011-2014, SE deprivation has become an independent negative prognostic factor.

Table 1. Hazard ratios for overall survival for patients diagnosed between 2011-2014 comparing to 2007-2010 stratified by socio-economical deprivation

	Hazard Ratio	p-value
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Decile 1-2	0.570	<0.001
Decile 3-4	0.860	0.289
Decile 5-6	0.807	0.088
Decile 7-8	0.717	0.003
Decile 9-10	0.812	0.075

220. Failure to achieve early disease response predicts inferior survival in newly diagnosed multiple myeloma

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Aim

The novel agents, the immunomodulatory drugs and proteasome inhibitors, are cornerstones of multiple myeloma (MM) therapy. While triplet induction regimens administering both novel classes concurrently are widely considered 'gold standard', the costs of such protocols are prohibitive for many healthcare systems.

It has been demonstrated that deeper response rates post induction correlate with improved survival in transplant eligible and ineligible patients. However, it is currently not known whether earlier stratification during standard induction, will identify patients in 'real-time' who are likely to have poor long-term outcomes and therefore may benefit from early escalation of therapy.

Methods

We conducted a retrospective review of all newly diagnosed patients with MM at Monash Health treated with bortezomib-based induction between January 2009 and December 2016. We have tested the hypothesis that failure to achieve at least a partial response (PR) following cycle two of induction predicts for worse overall survival and identifies patients in whom treatment escalation should be considered.

Results

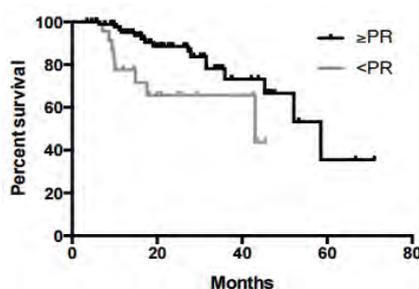
Of 115 patients included in the analysis, 92 patients (81%) achieved at least a PR following cycle two, while 23 (19%) did not. No significant differences were seen between these two groups at baseline, including traditional prospective risk factors. Failure to achieve at least a PR following cycle two was associated with inferior overall survival (median overall survival 58.5 vs 43 months, P=0.014) (Figure 1). There was no difference in the rate of subsequent ASCT between the two groups to account for this survival benefit, and this also appeared to be independent of current prognostic factors.

Conclusion

Based on these results, we would advocate for the incorporation of a stratification after cycle two into the design of future clinical trials for MM to determine if directed treatment escalation results in similar treatment efficacy to triplet regimens with both reduced toxicity and use of finite health resources.

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

Figure 1: Overall survival stratified by response at Cycle 2



221. A young female with extensive lytic lesions

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Introduction

Multiple myeloma with extensive lytic lesions is very uncommon in the young.

Case report

A 30 yrs female presented with breathlessness, fatiguability and abdominal pain of 1 month duration associated with loss of weight, and pain over multiple areas of the body. Gradually the patient developed dysphagia with deviation of the tongue. On examination severe pallor, bony tenderness and palsy of the left hypoglossal nerve were present. Investigations showed Hb 4.5 gm%, TLC 3640 /cu mm, platelet count of 1 lakh, ESR 55 mm, blood urea 10 mg/dl, creatinine 0.9 mg/dl, Sodium 138 and potassium 3.57 meq/l, ionised calcium 1.13 mmol/l, total protein 6.1 and albumin 3.2 mg/dl, bilirubin 0.5 mg/dl, ALP 164, and TSH 3.5. Peripheral smear showed pancytopenia with microcytic red cells. ANA was negative. Serum ferritin was 533.5 ng/mL, and ANA was negative. Xray of the skull showed multiple punched out lytic lesions. Serum protein electrophoresis showed presence of M band 0.25% in the gamma region. Serum immunoglobulin profile showed decreased levels of IgG, IgM, and IgA. Serum free lambda (light chain) was highly raised was 8520 mg/L. Bone marrow study showed increased plasma cells constituting 30% of marrow nucleated cells and plasmablasts, indicating plasma cell myeloma. CECT of abdomen and pelvis revealed multiple lytic lesions diffusely in all of the visualised vertebra, pelvic bones and ribs along with wedging of T8 vertebra. Contrast MRI of brain and spine showed multiple infiltrative lesions in skull and diffuse marrow infiltration in spine with wedge collapse of D8 body and a right paravertebral mass lesion. Serum beta 2 microglobulin was raised 4428 ng/ml. The patient was diagnosed as Multiple myeloma (lambda light chain) with ISS-II, with XII cranial palsy.

Conclusion

This rare case highlights widespread bony involvement in a young multiple myeloma.

222. Updated efficacy and safety of daratumumab-lenalidomide-dexamethasone (DRd) versus lenalidomide-dexamethasone (Rd) in RRMM: POLLUX

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Aim

To provide updated efficacy and safety data from POLLUX, a multicenter, phase 3, randomized study of DRd versus Rd in RRMM.

Methods

Patients with ≥ 1 prior line of therapy (LOT) were randomly assigned to Rd (25 mg PO lenalidomide on Days 1-21 of each Q4W cycle; 40 mg dexamethasone weekly) with or without daratumumab (16 mg/kg IV QW for Cycles 1 and 2, Q2W for Cycles 3-6, then Q4W until disease progression). Bone marrow samples were collected, and minimal residual disease (MRD) was assessed at 3 different sensitivity thresholds (10^{-4} , 10^{-5} , and 10^{-6}) using next-generation sequencing (ClonoSEQ™). PFS was the primary endpoint.

Results

After 17.3 months median follow-up, DRd vs Rd prolonged PFS (median: not reached vs 17.5 months; HR, 0.37; 95% CI, 0.28-0.50; $P < 0.0001$), with 18-month PFS rates of 76% and 49%, respectively. DRd increased ORR (93% vs 76%), rates of \geq VGPR (78% vs 45%), and rates of \geq CR (46% vs 20%) versus Rd ($P < 0.0001$ for all). MRD-negative rates were >3 times higher (31.8% vs 8.8% at 10^{-4} ; 24.8% vs 5.7% at 10^{-5} ; and 11.9% vs 2.5%, at 10^{-6} ; $P < 0.0001$ for all) with DRd versus Rd, and MRD negativity was associated with prolonged PFS at 10^{-5} . OS data are immature (HR, 0.63; 95% CI, 0.42-0.95), with 40 (14%) and 56 (20%) deaths in the DRd and Rd groups, respectively. Neutropenia was the most common grade 3/4 TEAE (53% with DRd vs 38% with Rd), and no new safety signals were reported. Updated data based on approximately 25 months follow-up will be presented.

Conclusions

DRd significantly improved PFS, ORR, depth of response, and MRD-negative rates compared with Rd. DRd maintains a favorable safety profile. These data support the use of DRd in patients with ≥ 1 prior LOT.

223. Biology of IMiD® Compounds

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Thalidomide, and the IMiD® immunomodulatory agents lenalidomide and pomalidomide, have established themselves as a backbone of myeloma (MM) therapy over the last decade. This is demonstrated by 3 FDA approvals for new anti-MM agents in the last 12 months which are based on combinations with lenalidomide. However, the mode of action of these agents remained unclear until recently when the protein cereblon as part of a ubiquitin ligase complex was identified as the molecular target of thalidomide.

Subsequent studies demonstrated that cereblon expression is also essential for IMiD compound activity against MM and that cereblon binding of IMiD compounds leads to proteasomal degradation of two transcription factors, IKZF 1 and 3, which are critical to lymphoid and plasma cell homeostasis. It was subsequently demonstrated that proteasomal degradation of these transcription factors is not only critical for direct anti-MM cytotoxicity but also the immunomodulatory activity of the IMiD compounds.

Differential substrate specificity of IMiD compounds when bound to the ubiquitin ligase complex is likely to be responsible for different clinical profiles within this class of compounds.

This form of indirect targeting describes a unique mode of action and a new pharmacological principle in drug development, allowing the inhibition of proteins which otherwise are not considered “druggable” targets.

224. Outcomes for Asian relapsed multiple myeloma patients treated with carfilzomib vs bortezomib: ENDEAVOR subgroup analysis

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Background: Carfilzomib is a selective proteasome inhibitor that is approved as a single agent and in combination with dexamethasone or lenalidomide/dexamethasone for relapsed or refractory multiple myeloma. This preplanned subgroup analysis evaluated the efficacy and safety outcomes in Asian patients with relapsed MM from the ENDEAVOR study.

Methods: Study design and endpoints have been previously reported (Dimopoulos et al. Lancet Oncol 2016).

Results: Of 929 patients randomized, 109 (11.7%) were of Asian ethnicity (Kd=54, Vd=55) from the JAPAC countries and received study treatment. The majority were from Japan (n=44; 40.4%) followed by Taiwan (n=24; 22.0%), Singapore (n=20; 18.3%), Republic of Korea (n=16; 14.7%), and Thailand (n=5; 4.6%). Efficacy and safety results are shown in the table.

Efficacy	Kd (n=54)	Vd (n=55)
Median PFS follow-up (months)	8.4	7.6
Median PFS (months) (95% CI)	14.9 (13.1-17.7)	8.8 (6.6-NE)
HR (95% CI)	0.57 (0.29–1.14)	
ORR, %	79.6 (66.5-89.4)	70.9 (57.1-82.4)
Odds ratio (95% CI)	1.604 (0.664-3.872)	
≥CR, %	9.3	1.8
VGPR, %	63.0	23.6
Safety	(n=55)*	(n=53)*
Grade ≥2 peripheral neuropathy, %	0	29
Serious TEAE, n (%)	20 (36.4)	23 (43.4)
Significant AEs, n (%)		
Cardiac failure	0	2 (3.8)
Dyspnoea	2 (3.6)	9 (17.0)
Hypertension	4 (7.3)	14 (26.4)
Renal failure	0	0
Dose reduction due to AE, n (%)	34 (61.8)	13 (24.5)
Treatment discontinuation due to AE, n (%)	7 (12.7)	8 (15.1)

*safety population. ORR, overall response rate; CR, complete response; VGPR, very good partial response; TEAE, treatment-emergent adverse event;

Similar patient incidence rates of AEs, ≥Grade 3 AEs, and ≥Grade 3 treatment-related AEs were observed between the Kd and Vd arms except for higher cardiovascular events and hypertension being observed in Kd arm.

Conclusions: In general, the efficacy and safety results from the Asian population analyses paralleled and are consistent with the results from the overall population of the ENDEAVOR study although the small sample size limits definitive conclusions regarding rates of AEs. For patients of Asian ethnicity, Kd therapy led to clinically meaningful improvements in PFS, ORR, and CR/VGPR versus Vd.

225. Cauda Equina Nodules: A Curious Case of Central Nervous System Myeloma

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We report the third known case of cauda equina nodules manifesting as central nervous system (CNS) myeloma. A 63-year-old man was admitted to hospital with unsteady gait and progressive bilateral lower limb weakness. He had a 7-year history of non-secretory multiple myeloma in the form of multifocal skeletal plasmacytomas that persisted despite therapy with locoregional radiotherapy, thalidomide and dexamethasone and subsequent autologous stem cell transplant. In the four years prior to his current presentation, he developed new multifocal skeletal and cutaneous plasmacytomas requiring additional treatment with radiotherapy, thalidomide and dexamethasone before commencing on triplet therapy with bortezomib, cyclophosphamide and dexamethasone.

On this admission, examination was significant for bilateral hip flexion weakness with absent reflexes of the lower limbs. Magnetic resonance imaging (MRI) of the spine revealed multiple new avidly enhancing cauda equina nodules at the L1-L3 region. Lumbar puncture was performed and cerebrospinal fluid (CSF) analysis confirmed the presence of a clonal plasma cell population consistent with CNS myeloma. MRI-Brain and positron emission tomography (PET) scan revealed only localised disease to the cauda equina. Serum electrophoresis did not reveal a paraprotein and his serum free light chains were normal. Bone marrow biopsy performed was also negative.

The patient was treated with targeted radiotherapy, intrathecal methotrexate and high dose intravenous methotrexate and cytarabine. However, repeat lumbar puncture and CSF analysis revealed persistent disease. Twice weekly intrathecal cytarabine, methotrexate and dexamethasone were subsequently administered until complete cytological response was achieved on CSF analysis. He was then commenced on maintenance lenalidomide and dexamethasone with no evidence of disease progression at 3 months.

226. Quantification of plasma Epstein–Barr virus DNA for monitoring of treatment response of plasmablastic lymphoma

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Introduction

Plasmablastic lymphoma (PBL) is a rare and aggressive type of non-Hodgkin's lymphoma. The pathogenesis is Epstein–Barr virus (EBV) related in most patients.

Method

Circulating EBV DNA level was measured at diagnosis and serially every 2 months using quantitated real-time PCR. Results were expressed as copies per millilitre of total EBV DNA calculated using a standard curve, and log scale interpretation was used.

Result

A 60-year-old gentleman presented with progressive bilateral parotid swelling and axillary lymph node enlargement for 1 month. Biopsy of axillary lymph node confirmed PBL. Further work-up showed that it was stage IIIA disease. Bone marrow biopsy was free of lymphoma. The patient was HIV negative.

He was treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) chemotherapy. Computerized tomography scan repeated after 3 cycles of therapy showed remission of the lymphoma. He then declined chemotherapy because of the side effects, and there was relapse of lymphoma with progressive enlargement of lymph nodes and parotid glands 2 months later. Patient was willing to continue chemotherapy, and CHOP therapy was given. But there was disease progression and the patient finally died of progression of the lymphoma.

The plasma EBV DNA levels showed good correlation between the viral load and the clinical and radiological responses. The level was 58,500 copies/ml at presentation and became undetectable after 3 cycles of CHOP chemotherapy. At relapse, the plasma EBV DNA increased again to 5,280 copies/ml. The patient did not respond to treatment, and the EBV DNA level further rose to 17,170 copies/ml, accompanying with clinical progression. Before death, the EBV DNA level remained elevated (12,225 copies/ml).

Conclusion

Measurement of EBV DNA is quantitative, sensitive, and simple to perform. It is more convenient and ideal for tumor surveillance than conventional radiological studies. Its use as surrogate biomarker for PBL is worth further exploration.

227. Unraveling CD45 in multiple myeloma through CRISPR/Cas9 gene editing

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Aim

CD45 (*PTPRC*) is a receptor-like protein tyrosine phosphatase ubiquitously expressed on haemopoietic cells that mediates B cell receptor signalling through Src family kinases (SFKs). In multiple myeloma (MM) loss of CD45 expression has been correlated with earlier disease progression and inferior treatment outcomes, but the underlying mechanism(s) remain unknown. Our previous *in vitro* studies demonstrating a 'metastatic' phenotype for CD45 negative MM were limited to human myeloma cell lines (HMCL) with variable CD45 expression but with significant underlying inter-cellular genetic heterogeneity confounding further evaluation. To overcome this, we have utilised CRISPR-Cas9 system to knockout (KO) CD45 from HMCL thus eliminating this background heterogeneity.

Method

Four HMCL with high CD45 expression (OCI-MY1, XG-1, TK2 and U266) were transduced with a lentiviral construct containing CRISPR-gRNA targeted to exon 9 of *PTPRC* and two single cell clones from each HMCL were propagated. Loss of CD45 was evaluated by flow cytometry, immunofluorescence and immunoblotting. Genotypic, phenotypic and transcriptional changes were identified by Sanger sequencing, modified Boyden chamber assays, growth assays and RNA sequencing.

Result

Loss of CD45 expression was confirmed by flow cytometry, immunofluorescence and immunoblotting. The inhibitory phosphorylation on SFKs (Lyn p-Y507) was significantly enhanced validating the absence of CD45 phosphatase activity in the KO cells but no significant changes in cell morphology, proliferation rate or cell cycling profile compared to wild-type cells were observed. OCI-MY1 CD45 KO cells demonstrated reduction in homing capacity towards bone marrow stromal cells (by 62%, p-value<0.0001). RNA sequencing identified 71 and 148 differentially expressed genes in OCI-MY1 vs OCI-MY1KO and XG-1 vs XG-1KO, respectively. Validation of these data is in progress to guide further phenotypic analyses.

Conclusion

We have successfully generated CRISPR KO models to investigate the loss of CD45 in MM. Further evaluation will identify molecular mechanisms and pathways regulated by CD45 in MM.

228. A retrospective study of the prognostic significance of immunoparesis at diagnosis in multiple myeloma patients

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Aim

Suppression of uninvolved immunoglobulins is reported in up to 85-90% of newly diagnosed multiple myeloma (MM) patients, and is associated with poor survival¹. However, whether treatment with novel agents or immunoglobulin replacement therapy modifies the adverse prognosis associated with immunoparesis remains unclear. This retrospective study aims to analyze the impact of various prognostic factors especially treatment with intravenous immunoglobulin (IVIG) in myeloma patients with immunoparesis.

Method

A retrospective audit of patients diagnosed with symptomatic MM between 1/1/07 and 30/6/17 in the Illawarra Shoalhaven Local Health District was conducted. Outcomes were compared between patients with suppressed uninvolved immunoglobulins at diagnosis and those with preserved uninvolved immunoglobulins. Outcome measures included overall survival (OS), progression free survival (PFS), incidence of clinically-significant infections and impact of IVIG use and/or anti-myeloma therapy on infection frequency and immunoglobulin levels.

Result

26/45 (58%) of myeloma patients had immunoparesis at diagnosis. 10 of 26 (38%) patients in the immunoparesis and 2 of 19 (10%) in non-immunoparesis group went on to receive IVIG therapy. There were 80 clinically-significant infections in the immunoparesis group compared with 57 in the non-immunoparesis group. Other patient characteristics were similar between the two groups except that a higher frequency of ISS stage I patients in the non-immunoparesis group was observed. There was no significant difference in median PFS between the immunoparesis versus non-immunoparesis group; at 33 versus 22 months (p=0.12) respectively. Median OS was not reached. Additional data with more patients and statistical analysis will be presented at the meeting.

Conclusion

Modern induction treatment with novel agents and IVIG replacement therapy may have the potential to overcome the negative influence of immunoparesis at diagnosis in symptomatic MM patients.

Reference

¹ *Kastritis E, Zagouri F, Symeonidis A, Roussou M, Sioni A, Pouli A, Delimpasi S, Katodritou E, Michalis E, Michael M, Hatzimichael E, Vassou A, Repousis P, Christophoridou A, Kartasis Z, Stefanoudaki E, Megalakaki C, Giannouli S, Kyrtsolis MC, Konstantopoulos K, Spyroupoulou-Vlachou M, Terpos E, Dimopoulos MA Greek Myeloma Study Group. Preserved levels of uninvolved immunoglobulins are independently associated with favorable outcome in patients with symptomatic multiple myeloma. *Leukemia*. 2014;28:2075–2079*

229. Patient reported outcome measures in MM: real-time reporting to improve care (methodology of My-PROMPT study)

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Aim

In Australia, despite improvements in diagnosis and care, the five-year survival for people with multiple myeloma (MM) is only 43%. MM is also associated with a high burden of disease which compromises patients' health-related quality of life (HRQOL). Although improved overall survival remains a major goal of cancer treatment, HRQOL is increasingly important to patients and clinicians. However, few data exist on optimal measures to improve QOL in patients with MM. Involving patients in their care using patient-reported outcome measures (PROMs) to enhance QOL warrants investigation.

Method

This substudy of the Myeloma and Related Diseases Registry (MRDR) leverages the infrastructure and recruitment of the MRDR to test the feasibility of using a HRQOL questionnaire for MM as an intervention by highlighting concerning results to clinicians. The MyPOS questionnaire was validated to capture HRQOL outcomes in MM, is specifically designed for the clinical setting, and covers areas that can be overlooked by patients and busy clinicians.

Patients in the intervention arm complete the questionnaire before 4 clinical visits: at baseline (close to diagnosis), and at 1, 6 and 10 months. Treating clinicians are given a summary of the MyPOS results before the clinic visits and encouraged to incorporate this in the patient's care. Patients in the standard care group complete the questionnaire only at baseline and 10 months to assess change in HRQOL from baseline for both groups. Patients and clinicians complete surveys on the intervention that will be used to assess feasibility for wider use.

Results/Conclusion

Recruitment has commenced and will be active at 4 sites. If feasibility of patient-reported outcome measures (PROMs) as an intervention is demonstrated, we anticipate progression to a larger study to assess health benefits of the intervention and its impact on PROMs.

230. Early Monoclonal Para Protein Reduction and Response in Multiple Myeloma (EMPEROR study)

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Aim: Bortezomib or bortezomib/doxorubicin therapy in relapsed/refractory myeloma and reduction of paraprotein has been associated with longer time to progression¹. This is a retrospective analysis of newly diagnosed MM cases(2010-16) where novel agents were employed for induction.

Methods: We documented demographics, PP isotype, baseline PP values, and post induction regimen including number of cycles. Patients were grouped according to the best response based on International Myeloma Working Group classification². Survival was calculated using the Kaplan-Meier method with start time (ie landmark) designated as the date of first PP measurement after cycle 1. Standard statistical methods were used to collect other variables.

Results: 44 patients with a median age was 67 years (range 41-85) were included in the study. The major PP isotype was IgG kappa. The median baseline PP value pre-induction was 35 g/l and light chain difference was 2892 mg/l (range 98-14388 mg/l). Majority of patients received VCD (25). The median percentage reduction in PP post first cycle was 59% with greater reduction of disease marker for light chain myeloma compared with those with intact paraprotein. The best response for majority of patients to induction was very good partial response (VGPR) in 23 patients. Of 21 patients with > 50% reduction of disease marker after first cycle, 15 patients achieved VGPR/CR (71%). Of 15 patients with < 50% reduction of disease marker, 6 patients had VGPR/CR. 3-year OS after first cycle of induction was 63%. No difference in OS was noted in both cohorts. Patients with > 50% reduction in disease marker appeared to be more likely to achieve at least VGPR compared with those with < 50% reduction (OR 3.6, P = .089).

Conclusion: Myeloma patients treated with novel agents showed rapid reduction of disease marker however no relationship between depth of response and survival was noted which suggests a difference in disease kinetics based on the type of disease marker in myeloma, which may have implications for treatment scheduling.

References:

1. Rapid early monoclonal protein reduction after therapy with bortezomib or bortezomib and pegylated liposomal doxorubicin in relapsed/refractory myeloma is associated with a longer time to progression. Shah J et al :Cancer :2011 Aug 15;117(16):3758-62

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231. Is prelysis a valuable flow cytometric method for improving minimal residual disease (MRD) detection?

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Aim: MRD by multiparameter flow cytometry (MFC) is a powerful prognostic indicator for many haematological malignancies. Sensitivity of MRD assays is frequently impeded by low total white blood cell (WBC) acquisition number. Prelysis methodology is a novel technique which may increase sensitivity. We aimed to compare sensitivity and expression stability of prelysis versus postlysis methodology in MFC for MRD detection in two common haematological diseases, in which MRD detection plays an important clinical role; chronic lymphocytic leukemia (CLL) and multiple myeloma (MM).

Methods: Bone marrow aspirate samples from patients with CLL or MM were analysed with MFC, using standard postlysis technique as well as a prelysis technique modified from the Euroflow method. The total number of WBC and MRD events were evaluated to compare sensitivity of both methodologies. Antigenic expression stability was assessed by comparison of mean fluorescence intensity (MFI) of key antigens in the target populations between postlysis and prelysis assays.

Results: Samples from 20 MM patients and 17 CLL patients were collected and included for analysis. Prelysis samples achieved significantly greater total WBC events than postlysis samples in both CLL and MM, and overall increased limit of detection for MRD (see Table 1). Eighty percent of MM patients tested achieved a level of 500,000 total WBC acquisitions with the prelysis method, compared to 20% with the postlysis method. Importantly, both physical properties as well as MFIs of key antigens in both background normal populations, and MRD/abnormal events (if >30 MRD events were present), were not significantly different between the assays.

Conclusion: Implementation of prelysis methodology in MFC increases overall WBC acquisition and sensitivity of MRD detection in CLL and MM, without compromising assay stability.

	Postlysis	Prelisis	P value
Multiple myeloma			
Total no. WBC events	256,386 (16,858 – 757,364)	1,526,092 (183,339 – 3,992,717)	<0.001
Total no. MRD events	7 (0 – 5647)	115 (0 – 46,344)	0.14
MRD %	0.004% (0% - 5.18%)	0.007% (0% - 10.26%)	0.65
Abnormal plasma cells/MRD – MFI (8 of 20 patients with >30 events)			
CD19	0.26 (-0.32 – 1.42)	0.25 (0 – 0.49)	0.67
CD38	53.48 (11.05 – 122.30)	47.84 (8.3 – 119.09)	0.75
CD138	99.96 (10.8 – 242.08)	33.94 (0.68 – 108.02)	0.11
CD45	1.36 (0.37 – 4.01)	0.76 (0.30 – 3.63)	0.59
CD27	1.65 (0.37 – 16.06)	2.53 (0.29 – 20.80)	0.78
CD81	1.16 (0.51 – 11.22)	1.16 (0.78 – 5.1)	0.63
CD56	25.08 (0.43 – 86.71)	22.47 (0.57 – 82.37)	0.95
CD200	7.05 (2.21 – 35.61)	10.67 (3.3 – 76.18)	0.36
Chronic lymphocytic leukemia			
Total no. WBC events	567,774 (319,180 – 1,446,321)	3,782,025 (462,090 – 8,891,400)	<0.001
CLL cells/MRD – MFI (12 of 17 patients with >30 events)			
CD5	4.15 (0.19 – 22.6)	6.5 (0.26 – 17.3)	0.87
CD19	21.7 (9.2 – 43)	18.6 (8.85 – 47.9)	0.89
CD20	2.35 (0.34 – 18.7)	1.74 (0.38 – 20.6)	0.81
CD22	7.95 (0.94 – 24.6)	8.2 (1.2 – 38)	0.96
CD43	3.2 (0.13 – 9.2)	1.6 (0.13 – 5.9)	0.41
CD79b	2.34 (0.47 – 137)	1.7 (0.17 – 137)	0.86
CD81	2.88 (1.5 – 13.6)	2.50 (1.9 – 18)	0.61

Table 1. Total WBC acquisitions and antigenic expression stability in postlysis versus prelysis methodology in MFC for MRD detection

232. Updated efficacy and safety of daratumumab-bortezomib-dexamethasone (DVd) versus bortezomib-dexamethasone (Vd) in RRMM: CASTOR

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Aim

To provide updated efficacy and safety data from POLLUX, a multicenter, phase 3, randomized study of DRd versus Rd in RRMM.

Methods

Patients with ≥ 1 prior line of therapy (LOT) were randomly assigned to Rd (25 mg PO lenalidomide on Days 1-21 of each Q4W cycle; 40 mg dexamethasone weekly) with or without daratumumab (16 mg/kg IV QW for Cycles 1 and 2, Q2W for Cycles 3-6, then Q4W until disease progression). Bone marrow samples were collected, and minimal residual disease (MRD) was assessed at 3 different sensitivity thresholds (10^{-4} , 10^{-5} , and 10^{-6}) using next-generation sequencing (ClonoSEQ™). PFS was the primary endpoint.

Results

After 17.3 months median follow-up, DRd vs Rd prolonged PFS (median: not reached vs 17.5 months; HR, 0.37; 95% CI, 0.28-0.50; $P < 0.0001$), with 18-month PFS rates of 76% and 49%, respectively. DRd increased ORR (93% vs 76%), rates of \geq VGPR (78% vs 45%), and rates of \geq CR (46% vs 20%) versus Rd ($P < 0.0001$ for all). MRD-negative rates were >3 times higher (31.8% vs 8.8% at 10^{-4} ; 24.8% vs 5.7% at 10^{-5} ; and 11.9% vs 2.5%, at 10^{-6} ; $P < 0.0001$ for all) with DRd versus Rd, and MRD negativity was associated with prolonged PFS at 10^{-5} . OS data are immature (HR, 0.63; 95% CI, 0.42-0.95), with 40 (14%) and 56 (20%) deaths in the DRd and Rd groups, respectively. Neutropenia was the most common grade 3/4 TEAE (53% with DRd vs 38% with Rd), and no new safety signals were reported. Updated data based on approximately 25 months follow-up will be presented.

Conclusions

DRd significantly improved PFS, ORR, depth of response, and MRD-negative rates compared with Rd. DRd maintains a favorable safety profile. These data support the use of DRd in patients with ≥ 1 prior LOT.

234. The role of autophagy in multiple myeloma disease progression

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Aim

Relapse and drug resistance occurs in all patients with multiple myeloma (MM) with many cases manifesting extramedullary disease (EM) with progression. What enables MM to survive anoikis and egress from the bone marrow is unclear. Autophagy has been shown to enable tumour cells to survive anoikis, and nutrient deprivation. We studied the possible role of autophagy in MM progression utilising two pairs of human myeloma cell lines (HMCL) representing intra-medullary (IM) and EM disease derived from the same patient: TK1 (IM) and TK2 (EM), and KMS12BM (IM) and KMS12PE (EM).

Methods & Results

Microarray, demonstrated that SQSTM1 (p62), a cargo recognising protein that participates in selective autophagy and amino acid sensing, was expressed at higher levels in TK2 than TK1 (fold change 9.01816, $p=0.0003$). In concordance, p62 protein expression was 7-fold greater in TK2 than TK1. Functionally, LC3II turnover (autophagic flux) was 2.9 fold greater in TK2 ($p=0.01$) than TK1. This was recapitulated in the KMS12BM-12PE pair (2.6 fold greater autophagic flux in KMS12PE). TK1 and TK2 growth kinetics under conditions of nutrient deprivation (up to 21 days) became statistically different after the 3rd day, with TK2 continuing to proliferate until day14 ($p=0.0046$). This proliferative advantage was abrogated by the use of an autophagy inhibitor (chloroquine [CQ]=40mM), that did not significantly change the growth of TK1. Similarly, TK2 (101% of control) and KMS12PE (90% of control) proliferation but not TK1 (35% of control) nor KMS12BM (52% of control) proliferation was maintained after 24h of glutamine deprivation. Finally, under these same conditions CQ impacted more significantly on TK2 proliferation and survival (52% reduction of proliferation and 46.33% increase cell death) than TK1 (35% and 0.59% respectively).

Conclusions

Autophagy offers a survival advantage to MM under nutrient deprivation. We hypothesise that enhanced autophagy may promote MM disease progression and EM dissemination.

235. ASPIRE/ENDEAVOR: update from randomized, phase 3 studies of carfilzomib in relapsed/refractory multiple myeloma (RRMM) patients

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Introduction: In RRMM, carfilzomib, lenalidomide, and dexamethasone (KRd) was superior to Rd in ASPIRE, and carfilzomib and dexamethasone (Kd) was superior to bortezomib and dexamethasone (Vd) in ENDEAVOR for the primary endpoint of progression-free survival (PFS) by independent review. We report safety and efficacy data after 6–7 months of additional follow-up.

Methods: ASPIRE and ENDEAVOR study designs have been described previously (Stewart, N Engl J Med 2015; Dimopoulos, Lancet Oncol 2016). Comparisons were per stratified log-rank test; data presented here are per investigator assessment.

Results: In ASPIRE and ENDEAVOR, 792 and 929 patients were randomised, respectively. Baseline characteristics were balanced between arms in both the ASPIRE and ENDEAVOR trials. Efficacy results are shown in the table.

	ASPIRE		ENDEAVOR	
	KRd	Rd	Kd	Vd
Median follow-up (months)	37.8	37.0	19.4	17.7
Median PFS (months)	26.1	16.6	17.6	9.4
HR (95% CI)	0.67 (0.56–0.80); <i>P</i> <.0001		0.53 (0.44–0.63); <i>P</i> <.0001	
18-month PFS rate (%)	64.5	46.6	48.7	23.9
Median TTP (months)	30.5	18.9	19.4	10.2
HR (95% CI)	0.62 (0.51–0.76); <i>P</i> <.0001		0.50 (0.42–0.60); <i>P</i> <.0001	
Median TTNT (months)	NE	24.3	26.1	14.5
HR (95% CI)	0.62 (0.50–0.77); <i>P</i> <.0001		0.49 (0.40–0.60); <i>P</i> <.0001	

HR, hazard ratio; TTP, time to progression; TTNT, time to next treatment; NE, not estimable

In ASPIRE, 16.8% (KRd) and 19.0% (Rd) of patients discontinued due to AEs. Grade ≥3 AE rates were 5.9% and 2.2% for hypertension, 3.9% and 1.8% for cardiac failure, and 4.6% and 5.4% for peripheral neuropathy for KRd and Rd, respectively. In ENDEAVOR, 15.8% (Kd) and 14.9% (Vd) of patients discontinued due to AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for Kd and Vd, respectively.

Conclusion: Consistent with the primary analyses, these results show that incorporation of carfilzomib into treatment regimens in patients with RRMM results in clinically meaningful improvements in PFS and a favourable benefit–risk profile.

236. Carfilzomib triplets are effective in relapsed refractory multiple myeloma

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Aim

We audited the outcomes of relapsed refractory multiple myeloma (RRMM) patients treated with carfilzomib triplet regimens at North Shore Hospital to assess their efficacy.

Method

Patients with RRMM, were treated with compassionate Carfilzomib 60mg on D1, D2, D8, D9, D15, D16 (except Cycle 1 20mg/m² D1, D2) IV over 30 minutes, Dexamethasone 20mg PO on carfilzomib days, and either cyclophosphamide 300mg/m² D1, D8, D15 (KCd), Lenalidomide 25mg PO D1-D21 (KRd) or Thalidomide 100mg PO Daily (KTd), for nine 28 days cycles followed by maintenance (reducing carfilzomib to D1, D2). Thromboprophylaxis was Aspirin with KCd, or dabigatran with KRd and KTd.

Results

Since May 2016, 24 patients(pts) were treated with KCd (n=20) KRd (n=3) or KTd (n=1). The median age was 68 yrs (range 51- 91 yrs). Patient received a median of 3 (range 1-7) prior regimens, included bortezomib 24 pts (100%) (median 9, range 1-11 cycles), thalidomide 23 pts (96%), lenalidomide 22 pts (92%) pomalidomide 5 pts (21%) carfilzomib 1 pt (4%) and autologous BMT 15 pts (63%). Three patients (13%) had t(4;14).

The median number of cycles is 4 (range 1-15). As of 4/7/17, 15 patients (60%) remain on treatment and 9 have discontinued, due to progressive disease (8), or intolerance (malaise). Best response in 23 assessable pts was CR 3 pts (13%), VGPR 1 pt (4%), PR 9 pts (39%), MR 4 pts (17%), SD 2 pts (9%) and PD 2 pts (9%). The overall response rate (at least PR) was 54%, and at least MR was seen in 71%. With a median follow-up of 8 months (range 1-14 months), 13 patients have progressed at a median of 7 months (range 1-13 months). Deaths due to progressive disease have occurred in 4 pts.

Conclusion

Carfilzomib triplets are useful therapy for RRMM.

237. Depth of response and minimal residual disease (MRD) with daratumumab-bortezomib-dexamethasone (DVd) versus bortezomib-dexamethasone (Vd): CASTOR

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Aim: To evaluate the depth of responses achieved with DVd in CASTOR.

Methods: Relapsed or refractory multiple myeloma patients who received ≥ 1 prior line of therapy were administered 8 cycles of bortezomib 1.3 mg/m² SC (Days 1, 4, 8, 11) and dexamethasone 20 mg PO (Days 1-2, 4-5, 8-9, and 11-12) \pm daratumumab (16 mg/kg IV QW in Cycles 1-3, Q3W for Cycles 4-8, then Q4W until progression). High cytogenetic risk (determined using next generation sequencing; NGS) was defined as having t(4;14), t(14;16), or del17p abnormalities. MRD status was determined using NGS at 10⁻⁴, 10⁻⁵, and 10⁻⁶ sensitivities in patients with suspected CR and at 6- and 12-months after first study dose. PFS was the primary endpoint.

Results: After 13.0 month median follow-up, rates of \geq VGPR (62% vs 29%; $P < 0.0001$) and \geq CR (26% vs 10%; $P < 0.0001$) were significantly improved with DVd vs Vd, respectively. MRD-negative rates were higher (18% vs 4% at 10⁻⁴; 10% vs 2% at 10⁻⁵; 4% vs 1% at 10⁻⁶; all $P < 0.01$) and MRD negativity was reached earlier with DVd vs Vd. MRD-negative status was durable and was associated with prolonged PFS versus MRD-positive status.

In high-risk patients, ORR (82% vs 62%; $P = 0.039$) and MRD-negative rates (14% vs 0% at 10⁻⁵; $P = 0.0018$) were increased with DVd vs Vd, and no MRD-negative patients progressed. Similar ORR (85% vs 64%; $P = 0.0003$) and MRD-negative rates (12% vs 2% at 10⁻⁵; $P = 0.0011$) were observed in standard-risk patients.

Conclusions: MRD negative rate was higher with DVd vs Vd and was rapidly achieved. MRD negativity was also achieved in high-risk patients receiving DVd. MRD negativity was associated with prolonged PFS and may provide long-term clinical benefit.

238. Non uremic calciphylaxis in setting of Polycythemia and Multiple Myeloma

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Aim

Non uremic calciphylaxis (NUC) associated with haematologic malignancy is a rare clinical entity. We present an 83 year old male who presents with non-uremic calciphylaxis, on a background of JAK2+ polycythemia vera and kappa light chain plasma cell myeloma.

Case Presentation

The patient presented with polycythemia in 2011, and subsequently underwent bone marrow examination and genetic testing confirming JAK2+ PV. He was initially treated with intermittent venesections and oral hydroxyurea to good effect. In 2017, he presented with a left lateral lower limb ulcer approximately 7.5x11.5cm. Biopsy showed extensive ulceration and necrosis, as well as numerous medium sized to small arteritis showing heavy calcification within their smooth muscle walls, consistent with calciphylaxis. He had normal renal function, normal calcium, vitamin D, and was not on warfarin. Further investigation revealed lambda free light chains 3200, kappa light chains of 13. Bone marrow biopsy showed 20% plasma cell involvement, consistent with plasma cell myeloma. He was commenced on sodium thiosulfate, lenalidomide, dexamethasone, and a short course of busulfan, which was ceased due to thrombocytopenia.

Discussion

Non uremic calciphylaxis is a rare dermatologic condition with a poor prognosis. Systematic reviews report only 36 described cases, due to a variety of etiologies including metabolic disease, and malignancy (including multiple myeloma). Mortality rates were reported at 52%, with sepsis being the lead cause of death. The pathophysiology of NUC is poorly understood, and may relate to metabolic abnormalities involving calcium and phosphate. Multiple myeloma and PV are both prothrombic states, and this may have played a role in this presentation. Sodium thiosulfate has limited evidence in the non-uremic setting. Treatment is largely supportive with wound care, and treatment of the underlying condition.

Conclusion

NUC is a rare disease, generally associated with metabolic derangements and malignancy. This is the first time it has been reported in the setting of an MPN and MM. Further studies are needed regarding pathophysiology, and optimal treatment.

239. Prolonged bone marrow aplasia with lenalidomide for multiple myeloma – a case report

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Introduction

Mild, but not severe¹, cytopenias are a common adverse reaction to lenalidomide, while secondary haematological malignancies are much rarer. We report a case of severe bone marrow aplasia which progressed to AML likely secondary to lenalidomide.

Case report

A 76-year-old man diagnosed with smouldering IgA kappa myeloma in 2010 progressed to active disease in 2011. PR was achieved with cyclophosphamide, thalidomide and dexamethasone. He was maintained on thalidomide for 2 years, but developed haematologic progression in November 2016 for which Lenalidomide 25mg (days 1-21) and low-dose cyclophosphamide and dexamethasone was prescribed.

The patient's history was significant for chronic fluctuating neutropenia (neutrophils $0.7-1.7 \times 10^9/L$) and brachytherapy-responsive prostate cancer 10 years prior. Historical BM biopsies did not show significant hypoplasia/MDS. He had no other known factors interfering with lenalidomide metabolism.

Two weeks later, follow-up FBC showed haemoglobin 116g/L, WCC $1.7 \times 10^9/L$, neutrophils $0.3 \times 10^9/L$ and platelets $44 \times 10^9/L$. BM biopsy showed a markedly hypocellular marrow. Tests for PNH and viral pathogens were negative. PSA was $<0.1 \mu g/L$.

Lenalidomide was immediately ceased. Pancytopenia worsened and was complicated by episodes of febrile neutropenia and per rectal bleeding. Regular G-CSF and transfusion support was required with up to twice-weekly transfusions.

The patient's blood counts gradually improved and transfusion independence was achieved 5 months later, ultimately achieving what appeared to be VGPR with Hb 95g/L, WCC $7.6 \times 10^9/L$, neutrophils $4.5 \times 10^9/L$ and platelets $34 \times 10^9/L$. Two months later, $>50\%$ blasts were detected in the patients PB and BM biopsy showed AML.

Conclusion

We describe a case of prolonged BM aplasia following 2 weeks of lenalidomide-based therapy, which progressed to AML. Risk factors for secondary malignancy included brachytherapy and lenalidomide. Consequently, we propose dose reduction of lenalidomide for those with reduced marrow reserve, weekly FBC monitoring when initiating therapy and consideration of the carcinogenic effects of lenalidomide, particularly in patients with other risk factors.

No conflicts of interest to disclose

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240. Weekly subcutaneous CyBorD induction is effective pre-autologous stem cell transplant in patients with Multiple Myeloma

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Aim

Cyclophosphamide, Bortezomib and Dexamethasone (CyBorD) is currently the most common induction protocol in multiple myeloma (MM) in Australia.¹ Weekly dosing of Bortezomib has similar response rates with reduced toxicity compared to twice weekly dosing.² Subcutaneous (SQ) Bortezomib has demonstrated non-inferiority in efficacy, reduced toxicity and improved ease of administration compared to the intravenous route.³

These findings have led to weekly SQ bortezomib dosing becoming the preferred method of administration CyBorD in Australia, including as induction for patients undergoing autologous stem cell transplant (ASCT). However there is a virtual absence of literature studying outcomes with this CyBorD variation in patients undergoing ASCT. To this end we present our five year single institution experience in this cohort.

Method

Data was retrospectively analysed for consecutive, previously untreated MM patients inducted with four cycles of weekly SQ CyBorD (D1,8,15,22 of 28 day cycle) prior to ASCT between January 2012 and January 2017. This weekly dosing was utilised: bortezomib 1.3mg/m² SQ, cyclophosphamide 500mg PO and dexamethasone 40mg PO. ASCT conditioning was melphalan 200mg/m². Response and relapse criteria were based on standard International Myeloma Working Group (IMWG) consensus criteria.

Results

Twenty-six patients were available for analysis (Aged 43 to 68, average 59). ISS distribution was ISS 1 23%, ISS2 31% and ISS 19%.

After four cycles of SQ weekly CyBorD induction, overall response rate (ORR) and very good partial response rate or better (\geq VGPR) were 88% and 40% respectively. Response rates post ASCT improved to ORR 96% and \geq VGPR 81%. At median follow up of 31 months the progression free survival and overall survival were XX% and 88% respectively.

Conclusion

Outcomes post ASCT after induction with SQ weekly CyBorD protocol were similar to findings in the literature with intravenous or twice weekly CyBorD induction protocols. This abstract provides evidence of efficacy of this increasingly utilised and convenient induction regimen in transplant-eligible patients.

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241. Introducing self-administration of subcutaneous Bortezomib at home for people with multiple myeloma

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Aim

The Waikato district covers a large demographic and rural area, and there continues to be increase in the population which is placing more pressure on tertiary health services to provide required care / treatment in a timely manner. Within our organisation we are resource limited, both in staff and space, in the outpatient chemotherapy area. The introduction of self-administration of Bortezomib has seen improvement both for patients, and within the service delivery arena. This has reduced patient's travel time, increased their input into their treatment and reduced use of chair time in the outpatient chemotherapy suite.

Method

To implement a change in our practice we investigated the stability of Bortezomib, developed patient education, a telephone patient checklist, and a protocol for the outpatient chemotherapy nursing staff. The option of self-administration is discussed with patients requiring weekly Bortezomib. Patients are taught the technique and safety of self-administration in the outpatient chemotherapy suite. When they are confident to do so they are given the following week's syringe with a pre-loaded dose to take home. A blood test is required the day before each dose is due. Once the chemotherapy nurses receive the blood results they phone the patient and complete an assessment to establish that it is safe to continue with the scheduled dose.

Result

20 Patients are currently enrolled on the program.

Patient's express greater satisfaction with the service and direct involvement with their treatment.

Nursing staff have provided positive feedback regarding the easy to follow format of the assessment.

There has been an improvement on departmental capacity

Conclusion

The introduction of self-administration of sub-cutaneous Bortezomib for patients with Myeloma has improved both service capacity and overall experience for patients.

There is no conflict of interest to disclose

242. The epidemiology of systemic amyloidosis in Queensland.

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Aim

Systemic amyloidosis is characterised by tissue deposition of an abnormal misfolded protein leading to organ dysfunction and death. There is no data on the epidemiology of systemic amyloidosis in Australia and international data comes from studies based on death certificate data. This study aims to describe the incidence and mortality burden of systemic amyloidosis in Queensland based on case ascertainment from histopathology reports.

Method

Laboratory information systems from Queensland public and private sector pathology laboratories were searched to identify cases of “amyloid” or “amyloidosis”, for the years 1999 to 2013. Certain forms of amyloid (e.g. lichen amyloidosis) were excluded. The presence of a clonal plasma cell population identified on serum or urine protein electrophoresis or free light chain assay was considered a surrogate marker for AL amyloidosis.

Case mortality status was determined via linkage to the National Death Index. Univariate and multivariate analyses were undertaken to determine the influence of age, gender, paraprotein status and rural area on survival.

Results

441 cases of systemic amyloidosis were identified during the study period. 64.2% of cases were male. The mean age at diagnosis was 66 years (SD 13 years). The kidney was the most common site of biopsy diagnosis (22.2% of cases). The majority of cases (69.2%) had AL amyloidosis. The estimated incidence was 0.86 cases per 100,000 persons per year (95% CI 0.76-0.97). Median survival was 2.44 years (95% CI = 1.94-3.20). Increasing age (HR = 1.05, $p < 0.001$, 95% CI 1.03-1.07) and abnormal free light chain assay (HR = 2.44, $p < 0.001$, 95% CI 1.62-3.68) were associated with worse outcome.

Conclusion

Systemic amyloidosis has an incidence in Queensland of ~ 10 cases per million, and is associated with reduced survival. Queensland estimates of incidence and age-adjusted mortality are consistent with previously published studies.

243. Dosing of rVIII-SingleChain results in low bleeding rates in paediatric patients treated with prophylaxis

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Introduction

The safety and efficacy of rVIII-SingleChain was investigated in the AFFINITY program in two pivotal and one extension study. Studies were designed to reflect real-world practice; dose range and regimen were at the investigator's discretion based on bleeding phenotype and FVIII treatment used prior to enrolment. Dose and schedule could be adjusted at any time during the study.

Aim

This analysis evaluated initial and final dose assignment and corresponding bleeding rates in paediatric patients (<12 years) receiving prophylaxis 2- or 3-times weekly.

Methods

Total and spontaneous annualized bleeding rates (ABR/AsBR) were determined for patients stratified by age group (0 to 6 and 6 to <12 years) and either initial or final dose assignment. This analysis included paediatric patients assigned to prophylaxis 2- or 3-times weekly (84% of the subjects on prophylaxis therapy) in dosing brackets of 20 to <30 IU/kg, 30 to <40 IU/kg and 40 to ≤50 IU/kg.

Results

In the final dose assignment, 24, 19 and 24 patients were assigned a dose of 20 to <30 IU/kg, 30 to <40 IU/kg, and 40 to ≤50 IU/kg, respectively. ABR and AsBR were lower in those receiving ≥30 IU/kg than in those receiving <30 IU/kg. Median ABR for the dosing brackets of 20 to <30 IU/kg, 30 to <40 IU/kg and 40 to ≤50 IU/kg was 5.61, 2.75 and 2.34, respectively; median AsBR was 2.10, 0 and 0, respectively. Similar findings were recorded for both 0 to <6 year and 6 to <12 year age groups.

Conclusions

Dosing of rVIII-SingleChain based on clinical bleeding phenotype results in low bleeding rates in paediatric patients treated with prophylaxis 2- or 3-times weekly. However, differences in ABR and AsBR suggest children receiving >30 IU/kg are more protected from traumatic bleeding than those receiving <30 IU/kg. As paediatric patients are a very active population, a starting dose of 30–50 IU/kg 2- or 3-times weekly might be more appropriate.

244. Autoimmune haemolytic anaemia as presentation of early stage lung cancer

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Introduction

Autoimmune haemolytic anaemia (AIHA) is a rare complication of non-haematological malignancy, with only a small number of case reports showing an association with lung cancer and usually with advanced, incurable disease. We present a case of warm AIHA leading to the diagnosis of early stage lung cancer with marked initial improvement in haemolysis with tumour resection.

Case Report

An independent 82 year old lady presented with symptomatic anaemia with a haemoglobin value of 56g/L and MCV 109 fL. Work-up revealed reticulocytosis (reticulocytes $230 \times 10^9/L$), raised lactate dehydrogenase (LDH 756 U/L) and total bilirubin of 49 $\mu\text{mol/L}$. Haptoglobin was suppressed at $<0.08 \text{ g/L}$ and direct antiglobulin test (DAT) was positive for both anti-IgG and anti-C3 confirming the diagnosis of predominately warm AIHA. A small (2g/L) IgG kappa paraprotein band was also detected. 1mg/kg oral prednisolone was commenced and CT neck to pelvis did not show lymphadenopathy or bony lesions but did detect a 25x12x17mm spiculated subpleural mass in the left lower lobe of the lung. PET-CT showed an FDG avid, localised lesion. Biopsy confirmed lepidic adenocarcinoma and lobectomy was performed with curative intent. Haemoglobin stabilised immediately post-operatively and haemolytic markers began to normalise despite rapid weaning of prednisolone, only to recur at 7 weeks post-resection.

Discussion

Our case highlights the importance of being aware of the rare association of non-haematological malignancy and AIHA, as making a cancer diagnosis early may allow for potentially curative treatment and has been reported to achieve resolution of haemolysis. The recurrence of haemolysis in this case after initial resolution is of interest from an immunological point of view and raises the question of whether microscopic residual tumour or a sustained immune process, is responsible – a question which will hopefully be answered with further follow-up.

245. A Case of Hereditary Hyperferritinaemia Cataract Syndrome with concurrent homozygous C282Y HFE gene mutation.

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Hereditary hyperferritinaemia cataract syndrome (HHCS) is a rare autosomal dominant disorder, characterised by an elevation of serum ferritin levels and early onset cataracts. Elevated ferritin levels occur with normal to low levels of iron and percentage transferrin without parenchymal iron overload. Phlebotomy results in iron-deficiency anaemia without reduction in the ferritin level.

Early onset bilateral cataracts are characterised by multiple nuclear and cortical lens opacities related to L-ferritin crystal deposition.

Mutation of the iron responsive element (IRE) of the L-ferritin (FLT) gene provides the molecular basis for the up-regulation of ferritin synthesis and subsequent ferritin deposition in tissues and fluid.

A 36-year-old woman presented with an incidental finding of an elevated ferritin of 1500ug/L. Further investigation revealed a homozygous C282Y HFE gene mutation. An MRI of the liver showed no evidence of iron deposition. Treatment with three monthly venesections was poorly tolerated and the ferritin levels remained in the order of 1200-1500ug/L.

The patient and her son had both noted deterioration in visual acuity, with microscopy demonstrating central and speckled cataracts consistent with ferritin deposition. Molecular analysis confirmed a single nucleotide substitution of the iron responsive element of the ferritin light chain (FTL:c.-168G>T).

HHCS should be suspected in cases of isolated hyperferritinaemia associated with early onset of cataracts in the absence of iron overload.

A small number of cases of HHCS described in the literature presented with concurrent HFE gene mutation heterozygosity. To our knowledge, concurrent HHCS and homozygous HFE gene mutations have not been previously described.

The only treatment required for HHCS is management of cataracts, given the absence of iron overload. Despite concurrent homozygous HFE gene mutation in the cases presented, there was no evidence of iron overload. Monitoring for iron deposition as well as family screening are proposed as ongoing management in these unique cases.

246. Comparison of methods for diagnosing alpha thalassaemia in a multiethnic population

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Aim

To evaluate three methods for the diagnosis of alpha thalassaemia in a routine Haemoglobinopathy laboratory

Method

Patients (n=113) were evaluated for alpha thalassaemia by the iLab ICT strip test and Multiplex PCR for the 7 common alpha gene deletions (-3.7; -4.2; FIL; SEA; MED; 20.5 and THAI). Hb H inclusion assessment by Brilliant Cresyl Blue stain and manual slide examination was also performed on 54 of these samples that met laboratory criteria (i.e. MCV<75 and MCH <25, no beta thalassaemia or Hb variant present).

Results

- 12 alpha⁰ results by PCR were all detected by strip and Hb H inclusion
- 12 homozygous alpha⁺ results by PCR were all detected by strip
- 16 heterozygous alpha⁺ results by PCR were detected by strip
- 14 positive PCR results were negative by strip and negative or not evaluated by Hb H inclusion
- 8 positive strip tests were negative by PCR
- 4 homozygous Hb E patients were all negative by strip and PCR

Results from the iLab α thal ICT strip test were supported by the PCR method and were more sensitive than the Hb H inclusion test. Some limitations to the procedure were noted.

Conclusion

The iLab α thal ICT strip test method provides a sensitive, rapid and simple screening test for alpha thalassaemia when compared to Hb H inclusion method. DNA testing will distinguish the reproductively significant alpha⁰ from homozygous alpha⁺, however it requires additional equipment and expertise, thus does not suit as a screening method in a routine laboratory.

247. An unusual case of latent Plasmodium Vivax infection in multiple members of the same family.

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Introduction

This is a rare case where multiple family members of family who has recently migrated from Pakistan presented to hospital over the course of two weeks with active malarial *Plasmodium Vivax* infections caused by reactivation of liver hypnozoites.

Case Review

A 7 year old girl presented to Emergency with fever and abdominal pain. On routine evaluation of her blood film, *P.Vivax* was identified. Subsequent testing of the girl's mother, who was present with the girl and also febrile, confirmed she too had an active *P.Vivax* infection. The family had migrated from Pakistan to Australia eight months prior and had experienced intermittent fevers during this time. The following day the father and brother were tested for malaria, both thick and thin films being negative. Subsequent testing of the father a week later, who had since become febrile, showed active infection of *P.Vivax* which was confirmed by PCR.

Discussion

P.Vivax is known to have latent stages caused by liver hypnozoites. It has been hypothesised that reactivation often occurs after exposure to another Anopheles mosquito or post infection of *P.falciparum*. As this genus of mosquito is not present in Australia, reactivation of this family must have been caused by another stimulus, possibly another febrile illness.

This is one of the many causes of positive malarial infections seen at our hospital. With the increase in migration to this area and the tendency to of *P.Vivax* to form hypnozoites form, in particular the long-latency phenotypes where there can be period of up to 8-9 months before the first symptoms of re-infection are seen, we expect to see these numbers to continue to increase.

"No conflict of interest to disclose".

248. A spherical puzzle: Massive haemolysis following cardiogenic shock

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Case history

A 63 year old man presented to the emergency department with central crushing chest pain and anterior ST elevation on electrocardiogram. Soon after his initial assessment, he developed asystolic arrest. CPR was commenced as the patient was transported to the catheter lab, wherein angiography demonstrated diffuse coronary artery disease. A stent was inserted, and an IABP instated. He was then transported to ICU with infusions of heparin, tirofiban and noradrenaline. Echocardiography demonstrated marked systolic dysfunction, at which point ECMO was deemed necessary. Within an hour of initiation, the haemoglobin had dropped from 142 to 57, with visible haemolysis. The blood film demonstrated new and marked spherocytosis, toxic change in the neutrophils and occasional fragments. All subsequent biochemistry was unevaluable. Coagulopathy eventuated with raised D-dimer, prolonged APTT and PT, and low fibrinogen, however the platelet count was, and remained normal.

In the following hours, lactate acidosis worsened, with numerous arrhythmias ultimately descending into asystole. The patient was pronounced deceased 24 hours following his presentation.

Discussion

Marked spherocytosis is associated with numerous processes including haemolytic processes, (autoimmune and alloimmune), drug related, clostridium toxaemia and burns. However, in this case, there was no evidence sepsis, infusion of incompatible blood products (the patient's group was AB+), or classically implicated drugs. Though intravascular devices (both ECMO and IABP) are known to cause haemolysis, it is more commonly seen in ventricular assist devices. Microangiopathic haemolytic anaemia may itself cause spherocytosis, however more characteristic fragments (keratocytes, helmet cells and crescents) were absent. Finally, any contribution of anaesthetic induction agents or accidental infusion of water may plausibly have contributed to the sudden change in this gentleman's blood film, and G6PD was as yet untested; however no cohesive diagnosis is evident to explain the *magnitude* of our findings.

249. Management of haemoglobin Barts hydrops fetalis syndrome with exchange transfusions

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Haemoglobin (Hb) Bart's hydrops fetalis syndrome (BHFS) results from deletion of all four alpha-globin genes. Whilst generally considered fatal in the antenatal period, rare patients are surviving into adulthood. Here we present a 24-year-old female with BHFS managed with regular exchange transfusions.

Case report

The patient was born at 35 weeks' gestation. She was homozygous for the South-East Asian two-gene alpha-deletions. Her initial haemoglobin was 7g/dL, with 86% Hb Bart's. She received three-weekly blood transfusions and iron chelation with deferoxamine. Her care was transferred to Texas, USA at age 8.

She underwent splenectomy age 14. This was complicated by recurrent venous thromboembolisms. Due to concerns of hyperviscosity precipitating further thrombosis, she only received transfusions every eight weeks, maintaining her haemoglobin between 10-12g/dL. Deferoxamine was changed to deferasirox. She returned to Australia age 23.

On her return, her haemoglobin was 10g/dL, with 51% HbH. Her functional haemoglobin was 4.9g/dL. She suffered severe lethargy and dyspnoea. Her ferritin was 1956mcg/L. She commenced fortnightly partial exchange with one unit of packed red cells. Four months later, she started automated red cell exchange transfusion by apheresis with 6 units of red cells. Within three months, her pre-transfusion HbH was 27%, haemoglobin 12g/dL, giving a trough functional haemoglobin of 8.7g/dL. Her symptoms significantly improved. Her ferritin was 1468mcg/L.

Discussion

BHFS is characterised by low functional haemoglobin and profound haemolysis. A standard transfusion program similar to that used for transfusion dependent beta-thalassaemia does not adequately address these issues. Regular exchange transfusions more effectively suppress erythropoiesis and improve functional haemoglobin. Whilst previous experience employing aggressive hypertransfusion regimens have proved successful at reducing HbH levels, this is at the cost of severe iron overload. Splenectomy should be avoided in these patients given the high risk of thrombosis. This case demonstrates the successful utilisation of regular exchange transfusions in the management of BHFS.

No conflict of interest to disclose.

250. A single-centre audit of antenatal haemoglobinopathy screening and the referral process for DNA analysis

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Haemoglobinopathies are a significant global health problem, and may become an issue in Australia with increased migration. Screening aims to identify 'at risk' females in order to provide education, genetic counselling, and reproductive choices pre-conception or in the early antenatal period. For alpha thalassaemia, severe clinical syndromes include HbH disease and Hb Barts hydrops fetalis.

Aim

To evaluate antenatal haemoglobinopathy screening at Melbourne Pathology in a cohort of pregnant women with results suggestive of alpha thalassaemia carrier state. Outcomes of DNA analysis in this cohort were assessed against red cell parameters to determine whether selective referral based on presumed risk would be feasible to reduce the burden of testing couples at low-risk of producing a child with a severe alpha thalassaemia syndrome.

Method

Retrospective review of 36 months of haemoglobin studies data and alpha gene mutational analysis. Inclusion criteria: pregnant females, age 15-45yrs, MCH <28pg, ferritin >30µg/L, HbA₂ <3.4% with no additional haemoglobinopathy.

Results

Melbourne pathology performed 33,054 haemoglobinopathy screens over three years; 29.9% were antenatal screens. 7,653 samples were referred for DNA analysis; 22.3% from pregnant females. Red cell indices for individuals with single-gene and two-gene deletions were significantly different ($p < 0.05$) for Hb, MCV and MCH. There was sufficient discrimination (lack of overlap of indices) to safely distinguish 2 gene from no or one gene deletion, but not one gene deletion from a negative finding.

Conclusion

In possible alpha thalassaemia carriers, selective referral for DNA analysis based on presumed risk for a clinically significant genotype was possible using red cell indices (Hb, MCV, MCH) as discriminators between single-gene and two-gene mutations. This could facilitate a new model of immediate referral of individuals at risk of a two-gene mutation, and referral of possible individuals with presumed single-gene mutations only after partner analysis.

251. Rapid, Sustained Reduction in Complement-Mediated Hemolysis with ALXN1210 in Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH)

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Background

ALXN1210 is a humanized monoclonal antibody designed for rapid, sustained inhibition of C5 with less frequent dosing.

Aims

ALXN1210-PNH-103 is a Phase 1/2, multicenter, open-label, dose-escalation study (NCT02598583), evaluating the safety and efficacy of 2 IV dosing regimens of ALXN1210 in PNH patients (pts) ≥18 y naïve to complement inhibitor therapy.

Methods

In this interim analysis, 6 pts in Cohort 1 (C1) received either 400- or 600mg IV induction doses followed by 900mg maintenance dose q4w; 7 pts in Cohort 2 (C2) received 600- and 900mg induction doses, followed by 1800mg maintenance dose q4w. The primary efficacy outcome was change in mean LDH. Other endpoints included changes in FACIT-Fatigue scores.

Results

Median duration of exposure was 5.6 mo(C1) and 4.6 mo(C2). Pts had high haemolytic activity at baseline(BL), with LDH 7-fold higher than the upper limit of normal (ULN). LDH decreased rapidly by the first evaluable time point (day 8); improvements were sustained throughout all dosing intervals. Mean percentage reductions from BL in LDH were 85.9% in C1 at day 169(n=6) and 85.2% in C2 at day 141(n=5); respective LDH mean (SD) values were 232(82) and 198(36) U/L. At day 169, FACIT-Fatigue scores were improved in C1 (28.7% [52.7%] from BL), and improved by 76.2% (70.3%) in C2.

No deaths, serious adverse events (AEs), drug discontinuations, or AEs leading to withdrawals were reported. All AEs considered at least possibly related to therapy resolved with ongoing ALXN1210 treatment.

Conclusion

ALXN1210 treatment resulted in rapid and sustained C5 inhibition. Mean LDH were reduced to <ULN in 4/6(67%) of pts in C1, and in 6/7(86%) of pts in C2 at the last evaluable time point. Thus, the lower dose may be inadequate throughout the monthly dosing interval for complete suppression of haemolysis, which is central to the morbidities of PNH.

This is an encore abstract. The original abstract was presented as a poster at the American Society of Haematology (ASH) Annual Meeting in December 2016.

252. Patient-Reported Outcomes and Healthcare Resource Utilisation Before and During Eculizumab Treatment For Paroxysmal Nocturnal Haemoglobinuria

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Background

Eculizumab, a humanized monoclonal antibody, is the only approved treatment of Paroxysmal Nocturnal Haemoglobinuria (PNH). The International PNH Registry (NCT01374360) is a prospective, multinational, observational study established to study the course of PNH, QoL, safety and efficacy of eculizumab.

Aims

Analyse patient-reported outcomes (PRO) and healthcare resource utilisation (HRU) before and during eculizumab treatment.

Methods

Patient assessment questionnaire (PAQ) data for patients commencing eculizumab after Registry enrollment and had data available as of August 1, 2016, were analysed. Patients had to have non-missing data on demographics, ≥ 1 recorded PAQ within 12 months prior to eculizumab initiation, and ≥ 1 PAQ recorded ≥ 6 months after initiation. Outcomes of interest included changes in QoL assessments (FACIT-Fatigue score; EORTC QLQ-C30 score, Karnofsky Performance scale), disease symptoms, HRU, and times patients missed worked over the most recently reported 6 months prior to and during treatment.

Results

229 out of 649 eligible patients from 4082 enrolled patients met selection criteria for analysis. Clinically meaningful improvement in FACIT-Fatigue score (≥ 4 -point increase) was reported by 53% of patients during eculizumab (mean change, 5.2 points). Clinically meaningful improvement (≥ 10 -point increase) was observed in EORTC QLQ-C30 mean scores for global health/QoL (mean change, 15.1), role functioning(16.3), emotional functioning(12.1), social functioning(13.9) and Karnofsky scale scores(8.4 points). PNH-related symptoms disappeared in 19–44% of patients who reported the symptom prior to eculizumab across all assessed symptoms except erectile dysfunction. HRU decreased for emergency room visits and missed work days, (incidence rate ratio [IRR] [95% CI], 0.33[0.20, 0.54] and 0.48[0.25, 0.93], respectively) and increased for healthcare provider visits and hospital admissions (IRR [95% CI], 1.47[1.22, 1.77] and 1.17[0.60, 2.27], respectively).

Summary/Conclusion

Eculizumab was associated with clinically meaningful improvements in PROs, including assessments of fatigue, global health status, patient functioning, and disease-related symptoms, and a decrease in emergency room visits and number of missed work days.

253. Establishing reference intervals through indirect sampling, and the effect of age on MCV and RDW

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Aim: Most laboratories do not establish local reference intervals due to time and cost constraints. In this study, we aimed to obtain reference intervals (focusing on mean corpuscular volume (MCV) and red cell distribution width (RDW) by utilising data already obtained through routine laboratory work. Effects of gender and age were examined.

Methods: The study was performed at a large private laboratory network in Victoria, Australia. Patient results were extracted from the laboratory information system during the period 1st June 2016 to 1st February 2017. Samples must have been processed at the central laboratory, as these patients were more likely to be outpatients from the community. Inclusion criteria included age 18 years or older, only one FBE test during the study period, serum creatinine <101umol/L for males and <81umol/L for females, ferritin >30ug/L if available. Patients were excluded if anaemic by WHO criteria (males <130g/L, females <120g/L), or have red cell count (RCC) >5.4x10⁹/L for males or >5.0x10⁹/L for females. Sysmex XE-2100 analysers were in use during the study period.

Results: The resulting cohort comprised 26152 males (median 54 years, range 19-98 years) and 32340 females (median 51 years, range 18-99 years). The overall reference interval for females and males was determined to be 82.0-98.0fL (the current range in use was 80.0-96.0fL). Tables 1 and 2 displays MCV and RDW reference intervals stratified by age and gender. A statistically significant correlation with age was found for MCV and RDW for both genders. There were no significant gender based differences.

Table 1 – MCV stratified by age and gender

Age (years)	n	Female	n	Male
		Reference interval fL (CI)		Reference interval fL (CI)
18-30	4941	81 (81-82) - 96 (96-96)	2783	82 (81-82) - 95 (95-96)
31-40	4962	81 (81-81) - 97 (96-97)	3580	81 (81-81) - 96 (95-96)
41-50	5673	82 (82-82) - 98 (98-98)	4724	82 (82-82) - 97 (97-97)
51-60	6679	83 (82-83) - 98 (98-98)	5996	83 (83-83) - 98 (97-98)
61-70	5610	83 (83-83) - 99 (98-99)	5296	83.4 (83-84) - 99(98-99)
71-80	3075	83 (83-84) - 99 (99-100)	2823	84 (83-84) - 100 (99-100)
81-90	1257	84 (84-84) - 101 (100-102)	892	84 (83-85) - 101 (100-101)
>90	143	82 (81-84) - 103.4 (102-111)	58	85.5 - 101.6

Table 2 – RDW stratified by age and gender

Age (years)	n	Female	n	Male
		Reference interval (CI)		Reference interval (CI)
18-30	4941	11.8 (11.8-11.9) - 14.6 (14.5 - 14.7)	2783	11.8 (11.8-11.8) - 14.2 (14.1 - 14.4)
31-40	4962	11.9 (11.9-12) - 14.6 (14.6 - 14.7)	3580	11.9 (11.9-12) - 14.5 (14.4 - 14.5)
41-50	5673	12 (12-12) - 14.9 (14.8 - 15)	4724	12 (11.9 - 12) - 14.6 (14.5 -14.7)
51-60	6679	12 (12-12.1) - 14.9 (14.8 - 15)	5996	12 (12-12) - 14.8 (14.6 - 14.8)
61-70	5610	12.1 (12.1 - 12.2) - 15.2 (15.1 - 15.2)	5296	12.1 (12.1 - 12.1) - 15 (14.9 - 15.1)
71-80	3075	12.2 (12.1 - 12.2) - 15.5 (15.3 - 15.6)	2823	12.3 (12.2 - 12.3) - 15.5 (15.4 - 15.8)
81-90	1257	12.4 (12.3 - 12.4) - 16 (15.7 - 16.4)	892	12.4 (12.3 - 12.4) - 15.8 (15.6 - 16.5
>90	143	12.2 (12.1 - 12.7) - 16.9 (16.2 - 17.7)	58	12.3 - 16.3

Conclusion

Our results are concordant with others reported in the literature. Indirect sampling can be a fast and cheap alternative method to obtain reference intervals, and allows analysis of large amounts of data that may uncover useful correlations.

254: Green neutrophil inclusions – a sign of impending death?

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Aim

The appearance of bright green neutrophil and monocyte inclusions have been implicated in impending death when seen in the blood film (Hodgson *et al* 2015).

This study was to determine the outcome of patients in the last six months across our pathology organisation when these inclusions were seen.

Method

Eight patients were identified and the clinical details, laboratory results and patient outcome were collated. Two film reviewers confirmed the presence of these inclusions in either the neutrophils or monocytes or both.

Results

The patients ranged in age from 24 to 73 years and presented with a variety of clinical conditions. These included sepsis, cardiorespiratory arrest, heatstroke, metastatic cancer and HLH. Of the eight patients identified with these inclusions, five died between one and 10 days of the inclusions being seen on the blood film (62%). This agrees with the series of 20 patients published by Hodgson (2015) where the death rate was 65% and the timeframe was between one and eleven days.

Conclusion

The appearance of these green inclusions is usually a sign of impending death and this is supported in our study. It was found by Hodgson (2015) that these inclusions were lipid-rich, and probably derived from lipofuscin-like material released from necrotic liver parenchymal cells. In the majority of our patients, acute liver injury was one of the presenting features. These inclusions should be noted and reported by morphologists to alert the treating clinicians of the severity of the patient's condition.

Reference

Hodgson TO *et al* 2015. Green neutrophil and monocyte inclusions – time to acknowledge and report. *BJH* 170: 229-235

256. Haemoglobinopathy Screening – A One Year Of Audit Of Tests Performed At Monash Pathology

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Aim

Monash Pathology conducted an audit of all requests for Haemoglobinopathy screening during 2016 to look at the changing patterns of haemoglobinopathies being presented and to anticipate future diagnostic issues.

Method

For the period Jan-Dec 2016, we reviewed 6114 samples on 6023 patients, all of which had HPLC performed on a Bio-Rad Variant II analyser, and follow up gel electrophoresis and molecular analysis as required.

Results

4177 patients had screening results which were within normal limits. 801 patients had abnormal findings (including alpha thalassaemia, beta thalassaemia, structural haemoglobin variants, double heterozygotes, isolated raised HbF, and other miscellaneous abnormalities). 549 patients had iron deficiency or results indicating that thalassaemia could not be excluded and required further DNA testing. 482 patients had no FBE results submitted so an interpretation was not provided. The results are discussed in more detail on the poster

Conclusion

The screening protocol is an effective program to assist in the detection of patients with haemoglobinopathies, This study has enabled us to determine the prevalence of the different types of haemoglobinopathy in our population. As this population becomes more diversified, more rare and complex haemoglobinopathies are being encountered.

258. Gelatinous Transformation of Bone Marrow – A Case Study

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A 19/F was admitted to Emergency Unit of Royal Prince Alfred Hospital in Sydney. She presented with Right Upper Quadrant pain, fever, cachexia, generally unwell. Multilobar pneumonia was diagnosed by Chest X-Ray. She had no known allergy. Full Blood Count revealed that she was pancytopenic. Biochemistry showed deranged electrolytes, LFT, Cortisol & T4. Haematologist was consulted and Bone marrow aspirate and trephine biopsy were performed. Microscopic examination revealed that the Bone Marrow was profoundly hypocellular with adequate fragments. The background staining was filled with amorphous material. No sign of malignancy. The Trephine was also sent for histology section. Microscopic examination revealed that the eosinophilic amorphous background was evident. The diagnosis of **Gelatinous Transformation of Bone Marrow** was established due to Anorexia Nervosa.[1]The Pathology of Haematology behind the Gelatinous Transformation of Bone Marrow [2] will be reviewed and other disciplines of medical sciences will be discussed during the oral presentation session.

References

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259. A critical review of Pneumocystis Jiroveci Pneumonia (PJP) in clinical haematology and implications for prophylaxis

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Aim

Known risk factors for PJP in haematology patients include long-term corticosteroids and lymphosuppressive chemotherapy but the evidence for prophylaxis in many regimens is limited. We examined cases of PJP over five years in clinical haematology to determine risk factors to update local guidelines.

Method

Discharge summaries from 2012-2016 were searched and positive PJP polymerase chain reaction (PCR) microbiology reviewed. Diagnosis of PJP was based on clinical, radiological and microbiological evidence with infectious disease confirmation. Prophylaxis guidelines during this period included allogenic and autologous transplants, dose-intense chemotherapy > RCHOP21, lymphocyte depleting regimes (e.g. with purine analogues) or corticosteroids equivalent of ≥ 20 mg of prednisolone per day ≥ 4 weeks.

Results

Fifteen cases were identified, 13 were PCR positive, and 2 diagnosed clinically (1 PCR negative on BAL, 1 without BAL). Eleven (73%) were in patients with lymphoma, 8 with NHL including one each of RCHOP q14 and q21 (both age >65) and higher intensity regimes in 6 (all age <65), 3 with Hodgkin lymphoma (HL) receiving ABVD (median age 25). The other cases included ALL, post allogenic transplant, myeloma and ITP. Risk factors included lymphopenia (<0.6) at PJP diagnosis (11 cases), high dose steroids (7) and age >65 (6). Ten cases occurred when local prophylaxis guidelines were not followed; the remaining 5 fell outside the guidelines, including all HL cases, and 2 NHL (both age >65). The median stay in hospital was 9.5 days with 5 intensive care admissions. All cases were treated with therapeutic cotrimoxazole, with 3 chemotherapy delays but no deaths due to PJP.

Conclusion

Previous guidelines do not cover a significant percentage of PJP cases. We have adjusted our guidelines to add prophylaxis in all lymphoma patients with lymphopenia (CD4<0.2 or L <0.6) at diagnosis and to consider prophylaxis in those >65 receiving full dose R-CHOPq21.

260. Patient evaluation of bone marrow biopsy with regards to logistics, experience and satisfaction (PEBBLES).

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Aim

Anecdotally, patients experiencing their first bone marrow biopsy have difficulty understanding the nature of the procedure, accessing the hospital site, as well as overall dissatisfaction with the process.

We aim to assess patient perceptions and satisfaction regarding the bone marrow biopsy process, before and after the implementation of an information pamphlet, in patients who present for their first bone marrow biopsy at a tertiary referral service, and its associated referring hospital.

Methods

Patients who are required to have a bone marrow biopsy for the first time will be identified by the haematology advanced trainees. Each patient be invited to complete a questionnaire at the completion of the bone marrow biopsy. The questionnaire will assess each patient's satisfaction regarding: explanation of the procedure, when and where to attend on the day of the procedure, instructions on post-procedural care, whether written instructions were provided and whether the written instructions were useful, as well as overall satisfaction with the procedure.

After 25 consecutive patients have been identified, a 2-page information pamphlet, including a map of where to attend and a time and date reminder, will be made available to referring haematologists. A further 25 patients will then be assessed using the same questionnaire.

Data collection will be de-identified and blinded using an opaque, sealed envelope.

Results

The initial 25 patients are nearing completion of collection. The following 25 patients will be collected over the coming 2 months. The results of this study will assess the utility of written instructions as well as identifying if there are aspects of care that require further changes to improve patient experience and satisfaction.

261. Developing a Matrix for Appropriate Pathology Ordering in Haematology Inpatients in a Tertiary Referral Hospital

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Background

Inappropriate pathology ordering generates unnecessary direct (financial) and indirect costs (e.g. Morbidity to patients). Haematology inpatients who are frequently receiving chemotherapy and/or have bone marrow failure are often subjected to frequent pathology ordering.

Aim

To develop a matrix for appropriate pathology ordering after performing a retrospective review of ordering in an acute haematology ward in a tertiary referral hospital.

Methods

The patient group selected comprised of those under Haematology Inpatient care for a minimum of 48 hours in a single 7 day period. A training run had been conducted the previous week and then two independent reviewers (and a third if required to provide a casting vote) reviewed all pathology ordering for appropriateness in the 7 day period. A range of typical blood count, coagulation, transfusion and biochemical tests were considered.

Results

36 patients were admitted and 32 were evaluable. Average age 66yrs (range 31-87), Length of stay average 17 days (2-55). Patients had a variety of malignant and non malignant diagnoses. 897 individual tests were requested, mean at 46 per patient. The overall agreement between the two reviewers was 77.4%, highest for electrolytes, urea, creatinine (EUC) and full blood count (FBC) 95% and lowest for INR and APPT (COAGS) at 50%. In tests where there was agreement, appropriateness was highest for FBC, EUC both at 98% but lowest for urate at 36% and COAGS at 33%. Group and save appropriateness was 97%, lactate dehydrogenase 91%, Ca/Mg/PO4 88%, liver function 86%.

Conclusion

These data indicate that on this acute Haematology inpatient ward there was a high level of appropriateness for most pathology ordering, however there is scope for improved efficiency with reduction in ordering of test such as COAGS and urate on a routine basis.

262. Patient outcomes of haematological malignancy requiring admission to intensive care – single centre experience.

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Aims

We aimed to investigate patient outcomes of haematological malignancy admitted to the intensive care unit. We compared ICU mortality between patients with haematological malignancy to those of the general ICU population, and identified patient factors that may predict poorer outcomes.

Methods

Retrospective audit of Department of Critical Care Medicine (DCCM) admissions at Auckland City Hospital between June 2014 and 2016. Cases were included if primary reason for admission was related to haematological malignancy. Data was collected from the ANZICS and Concerto database; which included: patient demographics, known haematological diagnosis, details of prior bone marrow transplant, reason for admission, number of organ failures (as per SOFA criteria) on admission and day 5. Outcomes collected were; the level of organ support received, whether treatment limitations placed, mortality during ICU stay, hospital stay and at 30 days, 90 days and 1 year following intensive care admission

Results

49 patient episodes were identified. Of this group, ICU and in-hospital mortality were 34.7% and 40.8% respectively. As compared to ICU survivors, patients who died in ICU were more likely to be younger (median age 47 vs 55.3; $p=0.0081$), female (58.8% vs 25%; $p=0.0194$), have a diagnosis of acute leukaemia (70.6% vs 40.6%; $p=0.0458$) and have a greater number of organ failures on admission (4 vs 2; $p=0.0142$). These included, coagulopathy on admission ($p=94\%$ vs 56%; $p=0.0062$), liver failure on admission (41% vs 13%; $p=0.033$), need for renal replacement therapy (35% vs 9%; $p=0.049$), and intubation during ICU stay (41% vs 13%; $p=0.033$).

Of note, prior bone marrow transplant (59% vs 31%; $p=0.0616$) and longer in-hospital time prior to ICU admission (15.6 vs 6.8 days; $p=0.0674$) may be associated with poorer ICU outcome, although not statistically significant.

Conclusion

ICU mortality in haematological malignancy remains high compared to a general ICU population. Our findings support early identification and consideration of admission would be beneficial in this vulnerable group of patients

263. Buffy the Fungus Slayer: Are Buffy Coats useful for treating infections in profoundly neutropaenic patients?

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Buffy coats can be used as supportive therapy in patients with life-threatening neutropaenia. With the advent of potentially curative intensive chemotherapy regimens used alone or in combination with stem cell transplantation, there has been an increase in patients with fungal infections during periods of prolonged neutropenia and subsequently, interest in the use of granulocyte transfusions to support these patients¹.

We report three patients who developed invasive fungal infections due to profound and persistent neutropaenia. Each patient was ordered buffy coat transfusions for varying periods. Two patients demonstrated little or no increment in white blood cell count as the result of the buffy coat transfusions, whereas one has showed small transient increments. All patients remained on treatment doses of antifungal medications throughout.

There are risks inherent in the use of buffy coats. As buffy coats have a very short expiry, they are typically administered after hours when there are less staff around to aid in the event of a complication. Granulocyte function deteriorates during storage and therefore the cells lose viability quickly. There is also a greater chance of alloimmunisation and febrile reactions due to the presence of leucocytes. Transfusion-transmitted diseases are also an important consideration in buffy coat transfusions. As buffy coats must be transfused as soon as possible after collection, infectious diseases testing has not been completed, so the risk is elevated. The cells are also stored at room temperature, leaving greater risk of bacterial contamination².

The decision to give buffy coat transfusions should be undertaken in a similar fashion to other blood product delivery, ensuring that the risks of transfusion do not outweigh the benefits. The decision to use buffy coats as a treatment choice must be considered carefully to ensure the patient is not exposed to added risk whilst already very vulnerable.

"No conflict of interest to declare"

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264. Reducing time-to-antibiotics in febrile neutropenia

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Introduction

Febrile neutropenia (FN) is the most common medical emergency in patients with haematological malignancy. Time-to-antibiotics (TTA) after the emergence of first fever is linearly correlated with prolonged hospital stay and 28 day mortality. The key performance indicator (KPI) for inpatients at the Austin Hospital is to receive antibiotics within one hour of first fever, which currently requires medical review prior to initiation.

Aim

To audit the TTA in haematology inpatients at a major teaching hospital in Melbourne as potential rationale to commence a nurse-initiated antibiotic protocol for patients at high risk of FN.

Method

Digital medical records of all haematology inpatients over a five year period (Jan 2011 to Dec 2015) were reviewed for episodes of FN, defined as a neutrophil count of $<0.5 \times 10^9/L$ and fever $>37.5^\circ C$. Exclusion criteria included patients who clinically had non-infectious causes of fever, absence of documented TTA and those who had antibiotics commenced prior to fever.

Result

280 patients who fulfilled the criteria were included in the review. The median TTA was 1 hour 23 minutes (range: 5 minutes to 20 hours). 70% of patients received antibiotics beyond the KPI of 1 hour and 29% beyond 2 hours. Delay in medical review was positively correlated with prolonged TTA but data also suggests an additional delay from review to time of antibiotic commencement. Further analysis of contributory causes and the impact this delay has upon patient outcomes is currently being undertaken.

Conclusion

This retrospective review revealed that most patients fell outside the one hour KPI for treatment of FN, with delayed medical review a major contributory factor. This has led to the proposed development of nurse-initiated, pre-prescribed antibiotic orders for high risk patients which aims to minimise such delays. This will be prospectively audited.

265. Urine cultures at onset of febrile neutropenia (FN) rarely impact antibiotic management in asymptomatic patients

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Introduction

Our unit has a focus on evidence-based approaches to the investigation of FN and have previously shown that routine CXRs are not generally useful. Urine cultures are also commonly performed in this context. However specimen collection may delay the administration of antibiotics and laboratory costs are not inexpensive. Moreover the impact of culture results on subsequent antibiotic management is not well documented in patients receiving protocol specified broad spectrum antibiotics. We assessed this latter issue in a retrospective review.

Aim

To evaluate the impact of urine cultures on the management of FN in haematology inpatients at a major tertiary hospital

Methods:

Records of all haematology inpatients over a 5-year period (2011-2015) were reviewed for episodes of FN (neutrophil count $<0.5 \times 10^9/L$ and fever $>37.5^\circ C$). For each episode, demographic data, urinary tract symptoms and signs (absence of which was termed 'asymptomatic'), urine culture results and any consequent change in antibiotic management were collected. A urine culture was considered positive if $>10^8$ organisms/L of a predominant uropathogen were detected. Empiric antibiotic therapy for FN consisted of IV tazocin or IV meropenem, gentamicin and vancomycin if systemically compromised.

Results:

433 patients who fulfilled the criteria were included in the review, of whom 365 (84%) had a urine culture. Cultures were positive in 7 of the 317 (2.2%) asymptomatic patients and in 9 of the 48 symptomatic patients (19%). Only 5 patients (1.4%) had a change in antibiotic management due a urine culture: 6.3% of symptomatic and 0.6% of asymptomatic patients respectively.

Conclusion:

Urine cultures rarely impact on antibiotic management in FN patients without urinary tract symptoms. As a result of this study we no longer perform urine cultures routinely in FN, restricting this investigation to symptomatic or catheterised patients.

266. Transfusion in intensive chemotherapy for acute leukaemia and stem cell transplantation: impact of a Western Australian patient blood management program

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Background

Little is published on patient blood management (PBM) programs in clinical haematology. In 2008 Western Australia announced a health system-wide PBM program with PBM staff appointments commencing in November 2009. Our aim was to assess the impact this program had on blood utilisation and patient outcomes in intensive chemotherapy for acute leukaemia or haematopoietic stem cell transplantation.

Study Design and Methods

A retrospective study of 695 patient admissions at two tertiary hospitals receiving intensive chemotherapy for acute leukaemia or undergoing haematopoietic stem cell transplantation between July 2010 and December 2014 was conducted. Main outcomes included pre-red blood cell (RBC) transfusion haemoglobin (Hb) levels, single-unit RBC transfusions, number of RBC and platelet (PLT) units transfused per admission, subsequent day case transfusions, length of stay, serious bleeding, and in-hospital mortality.

Results

Over the study period, the mean RBC units transfused per admission decreased 39% from 6.1 to 3.7 ($p < 0.001$), and the mean PLT units transfused decreased 35% from 6.3 to 4.1 ($p < 0.001$), with mean RBC and PLT units transfused for follow-up day cases decreasing from 0.6 to 0.4 units ($p < 0.001$). Mean pre-RBC transfusion Hb level decreased from 8.0 to 6.8 g/dL ($p < 0.001$), and single-unit RBC transfusions increased 39% to 67% ($p < 0.001$). These reductions translate to 1111 fewer RBC units and 588 fewer PLT units transfused with product cost savings of AU\$ 694,886. There were no significant changes in unadjusted or adjusted length of stay, serious bleeding events, or in hospital mortality over the study period.

Conclusion

The WA health system-wide PBM program had a significant impact, reducing blood product use and costs without increased morbidity or mortality in patients receiving intensive chemotherapy for acute leukaemia or haematopoietic stem cell transplantation.

267. Complementary therapy improves the patient experience in a group receiving intensive chemotherapy for haematological malignancies.

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The Bone Marrow Transplant Unit at Christchurch Hospital, New Zealand has a new initiative involving an energy-based bio-field modality, using a mind-body approach to support health and well-being.

Aim

This offers a supportive, complementary, hands-on intervention for patients undergoing inpatient treatment for haematological malignancies. The pilot project will evaluate the feasibility and effectiveness of delivering the Healing Touch Therapy sessions to patients twice weekly, by a team of eight trained practitioners.

Method

Individual patients are allocated a single practitioner who delivers sessions lasting 45-60 minutes. Pre and post treatment evaluations from these patients are being collected using a modified Functional Assessment of Cancer Therapy- FACT- questionnaire and a Brief Symptom Perception Tool.

Results

Preliminary results show that "Healing Touch" is well received by the patients. Patients report an improvement in well-being and relaxation including in anxiety and muscle tension. Of the 25 patient responses, so far, 13 found the sessions very helpful, the rest were somewhat or quite a bit helpful and only one patient, on one occasion, did not find it helpful at all. One particular set of patient evaluations over six sessions, showed an increasing benefit over time.

Anecdotal comments include; "Today was great, so relaxed I fell asleep." Male 28 years This has been a very enjoyable thing to be involved in, let's hope it keeps going." Male 46 years. "Leg restlessness mitigated since session" Male 24 years.

Conclusion

"Healing Touch" therapy continues to be offered to all inpatients and evaluation is ongoing. We are hoping to integrate complementary therapies throughout the haematology service.

268. Cytopenias in various combinations found in Complete Blood Counts performed at a Diagnostic Referral Laboratory, Karachi

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Aims

To get an idea of the frequency of different cytopenias in CBCs samples arriving at Dow Diagnostic Research & Referral Laboratory (DDRRL), for analysis

Methods

Samples for complete blood count from April to June 2013 were reviewed and the frequency of various cytopenias was sorted to get an actual picture

Results

A total of 2000 samples were received and about n =320(16.0 %) were found to have cytopenias either alone or in various combinations. The great majority 11.95 % had isolated cytopenias and amongst them 74.68 % had isolated anemia which was mainly hypochromic microcytic type and was more prevalent in female population suggesting that anemia is the most common isolated cytopenias, followed by isolated thrombocytopenia, while combined A+T (anemia + thrombocytopenia) was the commonest bicytopenia followed by T+A (thrombocytopenia & neutropenia).

Conclusion

Majority of cytopenias were seen in adult and old age group. Cytopenias were more in female population especially anemia as they are more prone to develop iron deficiency.

269. Cellavision = proficiency efficiency - improving the quality and participation of morphology proficiency

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Aim

To understand the requirements in setting up the Cellavision proficiency program to replace the current labour intensive program.

Methods

Currently, 45 Haematology scientific staff members in our network are monitored for morphology proficiency 4 times per year by using the RCPA morphology films. The individual results are manually collated and when the RCPA final reports are received they are scored accordingly. Scores below 80% require a follow up by the senior scientist.

Cellavision proficiency software

Slides were stained using the Sysmex SP-10 slide maker/stainer with a commercial stain and buffer. 200 haematologically interesting slides were de-coverslipped and sent to Sweden for Cellavision to scan and upload onto the proficiency software. The slides were then be accessed via a web based interface to confirm the classification of cell type by the software. In cases where the cells were unknown an experienced morphologist performed a manual classification. Participants were then able to access the slides and perform a manual classification of all cell types. Their individual classification is then compared to the experienced morphologist and a score is given.

Results

Of the 45 participants, 22 failed to meet the minimum participation of 2 morphology exercises per year (2016 data). 14 failed to return any exercise. There were 16 incidences of scores below 80%.

Of the 200 slides sent to Cellavision only 71 were scanned. Reasons for rejection were mainly due to staining quality, slide length and slide type. The stain was deemed too dark for the auto classification analysis and yielded too much artefact (which was dark stained RBC's). 25 more slides were prepared using an altered staining protocol and sent. 20 of these slides were successfully classified and used for proficiency testing.

Discussion

Before utilising the Cellavision Proficiency Software it is important to understand the slide and staining requirements prior to large scale scanning. The use of this program is currently being developed in our network.

270. Capture the Flag - Validation of Rules and Flagging on the Sysmex XN9000

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Aim: To ensure that the newly installed Sysmex XN9000 FBP analyser in conjunction with the laboratories original validation rules did not increase the amount of false negative blood films.

Method: Following the ICGH guidelines, 797 FBP samples were reviewed over a period of 3 months. Analyser and flagging data was compared with the independent morphology result. The cohort was split into 4 groups; true positive, false positive, false negative and true negative. Immature granulocyte flagging data (IG) was also reviewed separately.

Results

All rules and flags:

	Number	%	ICGH Ref %
True positive	145	18.2	11.2
False positive	82	10.3	18.6
True negative	535	67.1	67.3
False negative	35	4.4	2.9
Total	797	100	

IG flag

	Number	%
True positive	45	5.6
False positive	21	2.6
True negative	710	89.1
False negative	21	2.6
Total	797	100

Specificity = 97.1%

Sensitivity = 68.2%

PPV = 68.2%

NPV = 97.1%

Conclusion: False negative (all flags) of 4.4% is higher than the reference 2.9% although lower than the recommended 5%. This high number could be due to the high percentage of positive films that FSH haematology had compared with the reference (18.2% vs 11.2%). The majority of the false negatives (60%) were due to RBC morphology changes.

The utility of a haematology analyser to identify these RBC abnormalities also needs to be considered when reviewing the false negative rate. The known clinically significant RBC haematological abnormalities such as spherocytes and schistocytes (fragmented RBC's) were not missed although the numbers in this study were small 4 and 4 respectively.

IG flagging showed a lower sensitivity but a higher specificity when compared to published data (68 vs 88 and 97 vs 84 respectively). The reasons for this might be explained in published papers "selected" population.

271. A time saving approach to G-6-PD screening

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Aim

In the interests of improving the turn-around time and reducing the cost of G-6-PD screen results the BinaxNOW® G6PD screening kit was trialled at Fiona Stanley Hospital over the course of 4 months. The kit was compared to the then current screening test (a commercial dye reduction method).

Method

46 samples were run by both the BinaxNOW® G6PD screening test and the Trinity Biotech G6PD screening test. 16 G-6-PD deficient and 30 normal samples were run by both methods. Samples for the study were sourced from Fiona Stanley Hospital and samples referred from other sites to PathWest QEII for quantitative G-6-PD analysis.

All samples were collected into EDTA, stored at 4°C and tested within the recommended times for each method.

Results

The BinaxNOW® G6PD screening test concurred with the Trinity Biotech G6PD screening test in all but one sample which gave an equivocal compared to deficient result for the Trinity Biotech G6PD screening test. As equivocal results are reported as deficient and referred for G-6-PD quantitation this result could be considered in agreement by both methods. The overall sensitivity of the BinaxNOW® G6PD screening test was 100%.

	Normal	Equivocal	Deficient	Total
Trinity Biotech G6PD	30	1	15	46
BinaxNOW® G6PD	30	0	16	46

Conclusion

The results of the trial showed that the BinaxNOW® G6PD screening test is an easy to use and cost effective method to screen patients for G-6-PD deficiency. The introduction of this method has initiated improved turn-around times for results and a decrease in staff time dedicated to G-6-PD screening.

272. Considerable reduction in blood film review rate by the introduction of middleware solution.

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Aim

Analyser Management System (AMS) haematology middleware (Abbott Diagnostics, Australia) was implemented to streamline Full Blood Count (FBC) processing in a multi networked laboratory group.

Methods

Sydney South West Pathology Service (SSWPS) is a centralised network of laboratories covering Liverpool hospital (CD Sapphire analysers), Bankstown, Fairfield, Campbelltown & Bowral hospitals (CD Ruby analysers).

Liverpool morphology review rates were 33%. The Ruby analysers generate a higher morphology review rates (41%). On average 1400 FBC's are processed per day at Liverpool and 600 across the other labs. The group performs 543441 FBC a year.

AMS haematology solution was implemented in August 2016. Auto-validation and blood film review rules were reviewed and modified to meet the International Council for Standardised Haematology (ICSH 2009) recommendations. Reference ranges rules were set slightly wider than accepted normal ranges and delta checks of previous results with various times depending of parameter. AMS rules were extensively tested on both set of analysers to confirm functionality. Rules were approved by the director of haematology.

Results

Since implementation morphology review rates have decreased to 27% in the Sapphire laboratory (approx.20% reduction). Ruby laboratories decreased to 35% (approx.15% reduction). There are improved efficiencies at the FBC processing area; staffing levels required in morphology have reduced as a result of AMS proving that an efficient middleware has benefited the haematology network greatly.

Additionally AMS provides a back-up solution in the event of laboratory information system failure (LIS). Downtime no longer affects the quality of service.

Conclusion

AMS has decreased the blood film review rate, improved efficiency and provided a flexible algorithm along With the ability to function and report results to clinicians with LIS failure, overall we have gained a more robust system which benefits clinicians, patients and staff.

273. Timing of blood cultures in the setting of febrile neutropaenia

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Background

Febrile neutropaenia (FN) is a medical emergency in haematology patients requiring admission to hospital, investigations to exclude infection and broad spectrum antibiotics. Blood culture (BC) positivity rates are low in FN. Contaminant organisms are not infrequent and iatrogenic anaemia an important consideration.

Aims

We sought to determine positivity rate of BC in a cohort of FN haematology patients, determine the utility of collecting BC after 24 hours of commencing broad spectrum antibiotics, and to examine adequacy of piperacillin-tazobactam as initial empiric therapy.

Method

We obtained blood culture results on all haematology patients with febrile neutropaenia admitted to Prince of Wales Hospital for calendar years 2014 to 2016. Patient-episodes of FN were defined for each patient. BC positivity was recorded for first BC, all BC within first 24 hours and all BC beyond 24 hours of commencing broad spectrum antibiotics. Isolated microorganisms were tabulated and their antibiotic sensitivities to empiric antibiotic regimen reviewed. Descriptive statistics with statistical inference through confidence intervals to analyse dataset.

Results

In total 378 patients experienced 912 patient-episodes of FN. 4252 BC were collected including 1468 after 24 hours of commencing empiric treatment. The median, minimum and maximum number of BC collected per patient-episode was 4, 1 and 40 respectively. In 121 patient-episodes (13.2%) microorganisms were identified. When considering BC positive episodes (1) the first BC was positive in 80%, (2) in only 6 cases (0.7%) were BC negative in the first 24 hours but subsequently positive. The identity and sensitivity of the isolated micro-organisms will be presented.

Conclusion

In conclusions our data demonstrates that BC are an important investigation for FN. We found that collecting BC beyond 24 hrs of commencing broad spectrum antibiotics rarely identifies relevant micro-organisms. Not collecting BC after 24 hours on broad spectrum antibiotics would reduce costs of pathology and lessen iatrogenic anaemia.

274. De-mystifying RhD

Westhoff C

New York Blood Center

RhD is one of the most clinically significant blood group antigens important in routine blood transfusion and as a cause of hemolytic disease of the fetus and newborn. It is also associated with ambiguities when serologic D typing of RBCs, which can present as discrepancies and/or weak or variable reactivity in approximately 2% of samples. With the cloning of the RH genes in the early 1990's, we now understand why D typing is sometimes not straightforward, as the diversity of the *RH* locus greatly exceeds all estimates predicted by serology. Although one primary *RHD* gene sequence is found in all populations, more than 500 allelic variations have now been reported to cause weak and/or altered D antigen expression, and new alleles continue to be reported. The challenge now is to determine which of the numerous alleles encoding amino acid changes result in the absence of D epitopes, or alter D epitopes, and result in risk for clinically significant anti-D when exposed to conventional RhD. It might be assumed that only extracellular changes would prompt an immune response, but altered surface epitopes also result from intracellular changes. Observational studies indicate that patients with weak D types 1, 2, and 3, which represent the majority of altered RhD in Caucasians, are not a risk for clinically significant anti-D. This has resulted in the use of *RHD* genotyping for obstetrical patients to guide clinical decision making for administration of Rh immune globulin and for transfusion of RBCs. Additionally, it is increasingly appreciated that *RH* genes encoding amino acid changes contribute to alloimmunization despite Rh antigen matching by serology in patients with Sickle Cell Anemia. "Demystifying D" is now possible with *RHD* genotyping to apply modern genomic methods to transfusion medicine and to foster the application of genomic science to promote more personalized and accurate transfusion medical care.

275. RhD HDFN – the current state

Liley H

Due to effective blood typing of pregnant women and anti-D immunoprophylaxis, RhD HDFN has declined markedly in Australia and other countries with high health care resources. Fetal and neonatal management of all but the lowest risk cases should now be under the direct guidance of designated centres that manage enough cases to maintain sufficient experience.

Although the prevalence of RhD negative blood type is highest in European populations, the global burden of RhD HDFN is estimated to be about three quarters of a million cases annually, with high absolute numbers of cases in some regions with low prevalence of RhD negative blood type but high birth rates due to lack of availability of immunoprophylaxis. Zipursky and Bhutani (Seminars in Fetal & Neonatal Medicine (2015) 20;2-5) have estimated that annually, RhD HDFN causes 41,000 stillbirths, 90,000 neonatal deaths and 97,000 cases of severe hyperbilirubinaemia of which at least 48,000 will survive to develop kernicterus – tragic outcomes that should be nearly 100% preventable. RhD HDFN has been identified among 9 “Urgent global opportunities to prevent birth defects” (Kancherla et al. Sem Fetal Neonatal Medicine 2014 19:153-60).

Among affected fetuses, sonographic measurements (chiefly middle cerebral artery peak systolic velocity) has superseded measurements of amniotic fluid bilirubin for determining which fetuses need intervention. Intrauterine transfusion remains a lifesaving procedure for fetuses with severe anaemia, and is now most commonly performed into the umbilical vein (umbilical cord or intrahepatic). It can prevent fetal death, prolong pregnancy (with benefits including reduced neonatal mortality and morbidity, and improved neurodevelopmental outcomes), and reduce the need for invasive procedures such as neonatal exchange transfusion.

As an example of the success of fetal management, Mater Mothers’ Hospital is a large (>10,000 births per year) maternity hospital that is a referral centre for maternal-fetal medicine and neonatal care. Audit of Mater Blood Bank records for the years 2003-2016 inclusive identified 162 intrauterine transfusions administered to 80 fetuses of 72 mothers. Of the transfusions, three quarters were for haemolytic disease of the fetus and newborn (HDFN), mostly for RhD or other red cell antibodies eg anti-Kell. Neonatal exchange transfusions were performed 48 times between 2003-2016, in 42 cases for HDFN (41 Rh or Kell, 1 ABO), with no baby receiving more than one exchange transfusion. Since then the incidence of exchange transfusion has declined, with no exchange transfusions performed since 2013. To prevent the need for exchange transfusion, IVIg is used by some centres, although the only two randomised trials at low risk of bias find that it is ineffective.

Intrauterine transfusion does not obviate the need to monitor affected infants for weeks to months after birth for late anaemia. A clinical trial is underway to determine whether an erythropoietic stimulating agent reduces that risk.

276. Challenges in preventing immunisation

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RhD negative pregnant women who become immunised to the RhD antigen are at risk of having subsequent pregnancies affected by haemolytic disease of the fetus and new-born (HDFN) when the fetus is RhD positive. Routine postnatal and antenatal 'anti-D' immunoglobulin prophylaxis programs have decreased rates of maternal allo-immunisation from up to 15% before 1967 to as low as 0.18% today.

A key challenge in the prevention of HDFN is to provide sufficient 'anti-D' enriched plasma for 'anti-D' immunoglobulin production. To prepare 'anti-D', volunteer RhD-negative donors are recruited and receive injections of RhD-positive red cells to boost levels of 'anti-D' in their plasma. 'Anti-D' is both a rare and valuable resource and there is a need to conserve supplies.

There is a potential to reduce the demand on 'anti-D' supplies because, in Australia, women who are carrying an RhD-negative baby (36 to 40% of RhD-negative mothers) currently receive antenatal 'anti-D' injections but are not at risk of being immunised. Non-invasive fetal RHD genotyping of cell-free DNA in maternal plasma predicts the fetal RhD blood group status and, therefore, by targeting routine anti-D immunoglobulin prophylaxis for women carrying an Rh-D positive baby, can reduce the usage of 'anti-D'. The technology incorporates automated DNA isolation systems with real-time PCR technologies.

Nation-wide non-invasive fetal RHD genotyping screening programs for all RhD negative pregnant women have been introduced this decade in countries such as Denmark, the Netherlands, and Finland. Regional or local programs are provided in Sweden and France and elsewhere. These programs report high patient compliance (e.g. >97% Denmark, >98% in the Netherlands), high accuracy and a low false negative rate. Maternal blood samples are collected at 25 to 27 weeks gestation, or, for some programs, in second trimester.

Within Australia we conducted a pilot economic analysis comparing potential costs and outcomes for current universal 'anti-D' prophylaxis versus non-invasive fetal RHD genotyping targeted 'anti-D' usage. The model predicts genotyping to be near cost-neutral to the health system: additional cost of non-invasive RHD genotyping being offset by lower demand and use of 'anti-D'. Outcomes were sensitive to key variables such as costs for 'anti-D' production, genotyping and sample transport.

The mechanism by which anti-D prevents maternal allo-immunisation remains speculative. With the support of the Blood Service Rh donor program, and in collaboration with the Australian Institute of Bioengineering and Nanotechnology, we are endeavouring to characterise "anti-D" at the molecular level. The goal is to supplement donor anti-D plasma with a manufactured blended synthetic "anti-D" to meet the challenge of maintaining supply for the future.

277. Transfusion reactions: Classifying them beyond ruling out an ABO error

Fung M

University of Vermont Medical Center

Appropriately diagnosing a suspected transfusion reaction has implications for how a transfusion recipient is treated and what additional products they may receive, and whether or not a donor or other donor-related blood products may be used. Furthermore, proper categorization and characterization of transfusion reactions is a necessary element of communicating among providers and for aggregating individual transfusion reactions into useful datasets for analysis. A brief overview of the current Hemovigilance definitions for transfusion reactions will be provided, followed by a review of various suspected transfusion reaction case scenarios that will help reinforce certain concepts such as case definition criteria vs. imputability, or the relationship of signs and symptoms to the transfusion. Additional cases and data will be presented where classification of the suspected transfusion reaction is not as clear cut. Data will also be presented where the use of a computer algorithm was validated against test cases of transfusion reactions. There will be some emphasis on respiratory reactions associated with transfusions where this has remained an area of difficulty in the field of transfusion medicine.

278. sTandaRd issue trANsfusion versuS Fresher red blood cell Use in intenSive care (TRANSFUSE) – a randomised controlled trial

McQuilten Z

Monash University

Red blood cell (RBC) transfusion is a common intervention in critically ill adults admitted to an intensive care unit (ICU). RBC units may be stored for up to 42 days prior to transfusion, depending on the additive solution and local policies. During this period, red cells and their storage medium undergo a number of changes, including structural, biochemical and metabolic, which are collectively referred to as the 'storage lesion.' A number of observational studies have reported associations between transfusion of older RBCs with worse patient outcomes, including mortality. Due to underlying illness severity, critically ill patients may be more susceptible to potential adverse effects of the storage lesion. However, recent randomised trials in cardiac surgery, general hospital and critically ill patients have not found any benefit from transfusion of fresher compared with older or standard issue RBCs.

We aimed to determine whether transfusion of the freshest available RBCs decreased 90-day mortality in critically ill adults. We performed a multicentre, double-blind, controlled trial comparing transfusion of freshest available RBCs with standard issue (oldest available) RBCs in 5000 critically ill adults. The trial was conducted at 59 sites in Australia, New Zealand, Finland, Ireland and Saudi Arabia. The primary outcome was 90-day mortality and secondary outcomes included 28-day and 180-day mortality, persistent organ dysfunction combined with death at 28-days, days alive and free of mechanical ventilation and renal replacement therapy, new blood stream infection during ICU admission, length of ICU and hospital stay and febrile non-haemolytic transfusion reactions.

TRANSFUSE is the largest blinded study comparing transfusion of RBCs of different storage age performed to date. The final results will be presented.

279. Transfusion-transmitted infections – the impact of rare infectious diseases

Hoad V

Red Cross Blood Service

The transfusion-transmission (TT) risk associated with the typical transfusion-relevant viruses such as human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) is extremely low. There has not been a confirmed TT of these viruses in Australia since the introduction of donor screening by nucleic acid testing (NAT), which was implemented during 2000 for HIV and HCV and 2010 for HBV. Internationally and in Australia, blood services are moving away from risk mitigation strategies based on zero risk tolerance. Blood safety decision-making is increasingly being driven by risk-based frameworks such as that developed by the Alliance of Blood Operators (The Risk-Based Decision-Making Framework available at <https://allianceofbloodoperators.org/abo-resources/risk-based-decision-making/rbdm-framework.aspx>.) This approach considers blood safety decisions in the context of the wider health sector, and incorporates the concept of risk tolerance that allows for risk mitigation strategies that are 'as low as reasonably achievable' taking into account evidence, practicality, sustainability and cost. Reported TT pathogens in developed countries have changed from the traditional blood borne viruses to pathogens for which, to date, relatively few transfusion-transmitted cases have been reported. Less frequently reported transfusion-transmissible pathogens such as hepatitis E virus, Ross River virus, parvovirus B19, Japanese encephalitis virus, human T-cell lymphotropic virus (HTLV) and *Treponema pallidum* (syphilis) will be discussed in the context of risk tolerability and their relevance to recipients in Australia. The risks these pathogens pose to blood safety in Australia are very low, enabling a safe and sustainable blood supply.

280. Platelet transfusions in very low birth weight infants: Time for some evidence

Josephson C

Blood, Tissue, and Apheresis Service

This presentation will focus on platelet transfusions in very low birth weight infants with an emphasis on neonatal thrombocytopenia, prophylactic platelet transfusion thresholds, and therapeutic treatment for bleeding. The data or lack-there-of-data that supports or refutes these practices will be presented; as well as, differing guidelines from multiple countries around the world. Further, with the efficacy of the prophylactic platelet transfusion preventing bleeding in premature infants being questioned, and the increasing evidence for risks associated with platelet transfusion, such as increases in mortality, the time for research in this area is now more than ever an imperative. The platelet product, storage solution, and supernatant composition are the most likely contributors to the uninvited immunomodulatory and angiogenic effects experienced in the premature infants and will also be discussed.

281. Recent advances in the treatment of fetomaternal alloimmune thrombocytopenia

Ghevaert C

Cambridge Institute for Medical Research

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) remains a very serious condition of pregnancy driven by the formation of alloantibodies against paternally-inherited fetal Human Platelet Antigens (HPAs) potentially causing intracranial haemorrhage (ICH) in the fetus leading either to death or serious neurological disabilities. The last decade has seen a shift from the use of invasive intrauterine transfusions of antigen-negative platelets to safer immunomodulatory therapies based on IV immunoglobulin in order to prevent ICHs in affected fetuses. New diagnostic laboratory assays have been developed which are gradually entering clinical practice, including next generation sequencing. Although prediction of clinical outcome remains one of the main challenge in the management of these pregnancies, new evidence has emerged that antibody binding epitopes may provide useful guidance. Large screening studies have generated data that increase the weight of evidence in favour of universal screening for FNAIT. Novel therapies involving either blockade of maternal antibodies with recombinant modified “protective” antibodies or FcRn blockade have been shown to have great potential, not only in murine models but in proof-of-principle first-in-man studies. The concept of prophylaxis to prevent alloimmunisation against HPA-1a antigens has re-emerged and is now being explored in large human trials.

282. Platelet transfusions in patients with hypoproliferative thrombocytopenia

Crighton G
Royal Children's Hospital

Abstract not supplied

283. PBM's impact on patient outcomes and economic consequences - the WA experience

Towler S

Abstract not supplied

284. Massive Transfusion Protocols: It's more than just asking for and receiving a lot of blood

Fung M

University of Vermont Medical Center

Massive transfusion protocols (MTPs) have been implemented in many medical centers to facilitate the delivery of large quantities of RBC units, plasma units, and platelet units in fixed ratios to avoid dilutional coagulopathy in patients requiring transfusions approaching or exceeding an entire patient blood volume. However, effective implementation of such protocols include the need for clearly communicating the activation of, and subsequent termination of such protocols. How multiple units of blood products are verified and issued by the Blood Bank for delivery to the operating room or critical care unit, can impact the timeliness of massive blood availability. Having mechanisms to address non-protocol based request for blood products reduces the possibility of confusion and delay. Is there a role for computer-based physician order entry of massive transfusion protocol orders vs. verbal orders in this emergent setting? What are the implications of identifying a previously unidentified patient in the electronic medical record system while a MTP is in use? Some United States survey data on practices and impressions regarding the use of MTPs at trauma centers will be shared followed by a discussion, and further sharing of experiences and ideas among audience members. This presentation will not cover studies regarding optimum blood product ratios, but focus rather on effective implementation of these protocols.

285. FFP and high INR in liver disease

Miranda S

St Vincent's Hospital Sydney

Standard coagulation tests, including PT and INR are frequently used to assess haemostasis. In liver disease, an elevated INR is used to reflect an impairment in the synthetic function of the liver and is incorporated into disease prognostic scores. However, impaired protein synthesis affects both pro-coagulant and anticoagulant factors important in haemostasis. The PT/INR are useful in diagnosing deficiency of procoagulant proteins, but are insensitive at detecting decreased levels of the anticoagulants. Traditional dogma based on incorrect interpretation of high INR is that individuals with liver disease have a bleeding tendency and are “auto-anticoagulated”. However, there is growing consensus that despite laboratory tests suggesting a bleeding tendency, haemostasis in liver disease is rebalanced with a bias towards a pro-thrombotic state.

Peri-procedural transfusional support with fresh frozen plasma and other blood products are often employed with the aim of reducing perceived bleeding risk, in spite of a growing body of literature showing that prophylactic plasma transfusion based on baseline INR does not reduce bleeding in patients, and may in fact cause harm. Against the use of plasma infusions stands the knowledge that the resulting volume expansion contributes to increase portal vein hypertension, aggravates decompensation and increases the risk of bleeding and rebleeding from oesophageal varices. Of addition concern, epidemiological studies have suggested that patients with CLD have to greatest risk of TRALI.

In the setting of liver disease with high INR, thromboestography (TEG/ROTEM) offers a new and alternative global assessment of haemostasis including coagulation factor function, platelet contribution to clot formation as well as fibrinolysis. Real-time viscoelastic testing has proven a robust tool guiding blood product transfusion during liver transplantation and may prove useful for other invasive procedures in patients with chronic liver disease.

286. Intragam 10 Adverse Reactions: Is Pharmacovigilance Enough?

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¹*Fiona Stanley Hospital, Murdoch, Australia*

Aim

Comparison of a large tertiary Hospitals experience with newly introduced Intragam 10 and its predecessor Intragam P.

Method

Blood Star records were accessed to determine number of IVIg recipients. Then a review of the hospitals Haemovigilance datasheet was undertaken as well as submitted Adverse Transfusion Reaction Forms. Investigation into cause of ATR's related to Intragam 10.

Results

The Blood Star database showed that 153 patients were registered at the site to receive IVIg. 44% received Intragam 10 (n=68). Of these, 11 patients (13 ATR's) were documented during the review period. 82% of patients had previously been treated with Intragam P.

Symptoms ranged from severe, sudden onset headaches (11/13 ATR's), Back and Neck pain (4/13), Facial flushing (2/13) and nausea (2/13). The amount of product transfused to time of symptoms ranged from 15ml-350ml.

Treatment included: Paracetamol- 85%, Decreasing rate-38%, IV hydration- 23%, Increased Hydration at 2:1 ratio to IVIg- 23%.

Two patients (18%) required IVIg change to another product, all others received subsequent doses of Intragam 10, with IV hydration.

Conclusion

The increased concentration of the new Intragam 10 product appears to be the cause of the developed symptoms. In 82% of patients, the introduction of pre-hydration prior to the infusion +/- a 2:1 Normal Saline/ Intragam 10 ratio was sufficient in preventing the adverse reactions occurring.

287. Platelet transfusion is not associated with increased mortality or morbidity in patients undergoing cardiac surgery.

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Aim

Transfusion of platelets is critical in cardiac surgery and while there are guidelines for their use, there are concerns about potential risks. Our objective was to assess the impact of platelet transfusion on mortality and risk of thrombosis and infection in this patient group.

Study Design and Methods

A retrospective cohort study of all patients at St Vincent's Hospital Melbourne (SVHM) who underwent a first cardiac surgery procedure from June 2001 to June 2014. A propensity weighted analysis was performed to examine the association between intra-operative platelet transfusion and outcomes.

Results

During the study period 5233 patients met the study inclusion criteria, 531 (10.15%) received intraoperative platelet transfusion with a median number of 1 platelet unit (IQR 1-17) administered. Patients receiving platelets were older, had a higher body mass index (BMI) with lower rates of diabetes and dyslipidaemia and higher rates of infective endocarditis; they were more likely to have recent pre-operative myocardial infarction and unstable angina, and exposure to aspirin or clopidogrel. On univariable analysis, platelet transfusion was associated with increased 30-day mortality (2.4% vs 10.55%, $p < 0.001$), return to theatre for bleeding (3.23% vs 13.37%, $p < 0.001$) and rates of any infection (9.26% vs. 19.17%, $p < 0.001$). After adjusting for confounders, platelet transfusion was not associated with increased risk of 30-day mortality or infective complications. Platelet transfusion was however associated with higher rates of return to theatre RR=2.46 (CI 1.42, 4.04; $p = 0.001$) and a decreased risk of cerebrovascular accidents RR=0.24 (CI 0.11, 0.49; $p < 0.001$).

Conclusion

Platelet transfusion was not associated with increased mortality or infective complications following first cardiac surgery in this patient group. Further prospective studies are required to identify patients most likely to benefit from platelet transfusion.

288. Granulocyte infusions: New Zealand experience.

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Background

Studies have shown granulocyte transfusions may be beneficial and potentially life-saving in severely neutropenic patients with severe systemic infections¹⁻⁵. New Zealand Blood Service (NZBS) has a policy for provision of granulocytes⁶ to New Zealand's District Health Boards (DHBs).

Aim

We set out to explore utilisation of granulocyte transfusions in New Zealand. The variables studied include demographics of the recipients, the clinical settings in which granulocyte transfusions were employed, the methods of collection of granulocyte products and clinical outcomes.

Method

Patients who had received granulocyte transfusions between 2006 and 2016 were identified from the New Zealand blood management system, eProgesa. Eight Transfusion Nurse Specialists working in eight DHBs reviewed the transfusion and clinical records.

Result

45 patients received granulocyte support for a total of 263 days. The median age was 16 years (range 0 – 74 years). The male to female ratio was 2:1. The indication for granulocyte transfusion was sepsis in all recipients. 79% of recipients had an underlying haematological malignancy with 50% having acute leukaemia. Others had aplastic anaemia, solid tumours, chronic granulomatous disease, or severe sepsis with no known predisposing condition. The median baseline neutrophil count was $0.19 \times 10^9/L$ (range 0-15.05) with 66% having severe neutropenia (defined as $<0.5 \times 10^9/L$). The median neutrophil count on the last day of granulocyte infusion was $0.55 \times 10^9/L$ (range 0-49.69). 56% had persisting severe neutropenia at the end of therapy. The median duration of support was 3 days (range 1-32). 68% of granulocyte support was via apheresis, the remainder whole-blood derived. The survival outcome was available on 44 of the 45 patients. 18 (41%) survived the acute illness.

Conclusion

Granulocyte transfusions were most frequently given to patients with an underlying haematological malignancy. The relatively low utilisation rate of granulocyte transfusions may reflect clinicians' attitude towards the benefit of such interventions and there may also be cost implications.

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289. Implementation of ROTEM[®] guided transfusion in a cardiac surgery unit

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Aim

To assess the impact introduction a ROTEM[®] guided transfusion algorithm has had on blood product use in a major cardiac center. Secondary outcomes were change in incidence of reoperation for bleeding and calculated cost from transfusions.

Method

This single center study included all patients undergoing cardiac surgery from April 2015 to December 2016. Data was collected on the use of packed red blood cells (RBC), fresh frozen plasma (FFP), platelets and cryoprecipitate, and secondary outcomes. The implementation period from arrival of the ROTEM[®], to the development and education around a local algorithm took nine months. Data was compared for the six months prior to the arrival of the ROTEM[®] machine, and the six months after implementation of the ROTEM[®] based algorithm. Data was analysed using Chi-square for categorical variables, and Mann Whitney U-test for independent, non-parametric data.

Results

We included 435 patients; 204 in pre-ROTEM[®] implementation, and 231 in post-ROTEM[®] implementation group. Introduction of the algorithm has resulted in a statistically significant reduction in individuals requiring FFP (43/ 204 (21%) vs 26/ 231 (11.2%), $\text{Chi}^2 = 7.8$, $P=0.005$), and pooled platelets (61/204 (30%) vs 44/ 231 (19%), $\text{Chi}^2 = 4.24$, $P=0.04$). An increase in individuals requiring cryoprecipitate was anticipated by the new algorithm, but did not occur (12/240 (6%) vs 16/231 (7%), $\text{Chi}^2 0.16$, $P=0.67$). There was no statistically significant change in the transfusion of RBCs (103/204 (50%) vs 95/231 (41%), $P=0.05$). A reduction in the number of surgical cases re-explored for bleeding (6/204 (3%) vs 3/231 (1%), $P=0.23$) and the median cost of transfusion (\$803.88 to \$401.94, $P=0.12$) occurred. Neither was statistically significant, but our estimates of the magnitude of these reductions would be clinically significant if confirmed in a larger sample. We recommend further data be sought in this regard.

Conclusion

These results support ROTEM[®] guided transfusion in the management of bleeding patients in cardiac surgery, and a shift away from transfusions guided by standard coagulation tests.

290. RBC alloimmunisation is associated with autoantibodies development and increased RBC transfusion requirements in myelodysplastic syndromes

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Aim: Up to 90% of myelodysplastic syndrome (MDS) patients require red blood cell (RBC) transfusions. However, literature addressing RBC alloimmunisation in MDS is limited. This study evaluates the incidence and clinical impact of RBC alloimmunisation in 817 MDS patients enrolled in the South Australian-MDS registry.

Method: Cumulative incidence (CI) of alloimmunisation was analysed by competing-risks regression using the Fine and Gray method. Factors associated with RBC alloimmunisation were investigated using random survival forest (RSF), recursive partitioning (RPART) and competing risk regression analysis.

Result: Median age of the 817 patients was 73 years, and 66% were male. CI of RBC alloimmunisation was 11%, with disease-modifying therapy being associated with a lower risk of alloimmunisation. Alloantibodies were most commonly directed against antigens in the Rh and Kell systems. Though 73% of alloimmunised patients developed alloantibody during their first 20 RBC units, total units transfused were significantly higher in alloimmunised compared to non-alloimmunised patients (90±100 vs. 30±52, p<0.0001; Fig 1A). A RSF analysis showed that in addition to the number of RBC units, RBC transfusion dependency (RBC-TD) status, treatment type and age were also important predictors of alloimmunisation. Furthermore, RPART suggested that 46% (39/84) patients who were RBC-TD had a higher risk of developing alloantibodies within first 20 units of RBC transfused. RBC transfusion intensity was significantly increased following alloimmunisation (2.8±1.3 vs. 4.1±2.0, p=0.002; Fig 1B). Autoantibodies were detected in more alloimmunised than non-alloimmunised patients (65% vs. 18%, p<0.0001; Fig 1C) with 80% of autoantibodies detected within 5-months of alloimmunisation.

Conclusion: This study characterises alloimmunisation in a large statewide cohort of MDS patients and demonstrates a significant increase in RBC transfusion requirement following alloimmunisation, most probably due to development of additional alloantibodies and autoantibodies, resulting in subclinical/clinical haemolysis. Strategies mitigating alloimmunisation risk are critical for optimising RBC transfusion management.

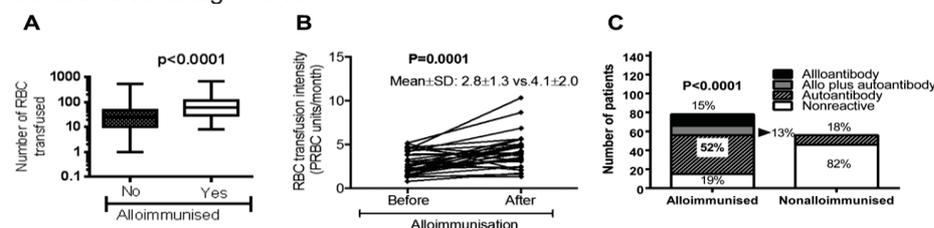


Fig 1. Alloimmunisation is associated with autoantibody formation and increased RBC transfusion requirements: (A) Total number of RBC units transfused were significantly higher in alloimmunised patients compared to non-alloimmunised patients (p<0.0001). (B) RBC transfusion intensity is significantly higher following alloimmunisation. (C) Autoantibodies were detected in significantly higher number of alloimmunised patients as compared to non-alloimmunised patients (65% vs. 18%; p<0.0001).

292. Pre-op haemoglobin optimisation algorithm and its effects on transfusion rates in elective orthopaedic surgical patients

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Aim: To improve the care and reduce the rate of transfusion of elective orthopaedic patients by reducing the risk of attending with pre-existing anaemia.

Method: An algorithm was developed for patients attending pre-admission clinic for major elective orthopaedic surgery which described the investigation and management of haemoglobin and iron levels.

Haematology, anaesthetics and gastroenterology were involved in the development of the algorithm to ensure appropriate follow up and testing of patients.

Data was collected to assess the use and effectiveness of the algorithm and compared with historical data.

Results

Orthopaedics	2012 N=231 (%)	2015 N=184 (%)	2016 N=205 (%)	2017 N=22 (%)
No. patients anaemic	21 (9)	26 (14)	16 (8)	2 (9)
No. patients with low ferritin	NA	6 -29 tested (21)	27 -194 tested (14)	5 -22 tested (23)
Total no. patients with either	21	27	38	6
No. patients who received treatment	NA	9 -8 received Fe infusion	22 -19 received Fe infusion	3 -3 received Fe infusion
Use of tranexamic acid	NA	78 (43)	131 (64)	14 (64)
Transfusion rate	80 (35)	32 (17)	24 (12)	4 (18)
Single unit transfusions	12/80 (15)	7/32 (22)	8/24 (33)	1 /4 (25)

Transfusion rate in 2012, when the algorithm was first introduced, was similar to audit data from 2009 when the rate was 33%.

At the end of 2015 routine ferritin testing was introduced to determine if we were missing iron deficient patients who were not yet anaemic, previously only anaemic patients had a ferritin added. Routine testing of ferritin continues.

Conclusion: Transfusion rate has decreased over the length of the project but cannot be attributed to the algorithm alone. Over the same time period there has been an increase in tranexamic acid use and single unit transfusions. This algorithm will be used with other surgical groups.

293. A revealing discrepancy between ordering and use of fresh blood products in Cardiac Surgery

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Aim: To audit the fresh blood product requests associated with coronary artery bypass graft (CABG) surgery and primary aortic valve replacement (AVR) surgery, and to correlate this with eventual transfusion of these components, with the goal of identifying potential product wastage and financial loss.

Method: A single centre, retrospective study of CABG and AVR surgery performed over the 6 month period December 2016 to May 2017. The ordering of fresh blood products was collected from the transfusion medicine log book. Transfusion records were extracted and filtered by patient to blood use in the operating theatre and ≤ 6 hours post-operative. The difference between ordered blood products and transfused blood products were classified as "Not used" rather than wasted, as some re-entered circulation.

Result

	Primary CAGS: 50 cases					Primary AVR: 35 cases					
	Ordered	Used	Not used	Not used %	CT Ratio		Ordered				
	Used	not used	Not used (%)	CT Ratio							
RBC	52	16	36	69%	3.25	RBC	30	6	24	80%	5
Platelets	37	18	19	51%		Platelets	41	17	17		24
	59%										
FFP	8	5	3	37%		FFP	47	38	9	19%	
Cryo	13	4	9	69%		Cryo	57	51	6	11%	

The results demonstrate high ordering patterns when compared to usage particularly for red cells, platelets and cryoprecipitate (CAGS only). The high Crossmatch-Transfusion ratio of 3.5 and 5 for CABG and AVR surgery respectively is much higher than the benchmark of 1.5-2.5 (Steven M. Frank, 2013).

CAGS AVR

Product transfused	Number of patients transfused	Product	Number of patients transfused
Red cells	4/50 (8%)	Red cells	4/35 (13%)
Platelets	16/50	Platelets	7/35
FFP	3/50	FFP	7/35
Cryo	2/50	Cryo	7/35

Patients not transfused 30/50 (60%) Patients not transfused 23/35 (65%)

Despite these procedures being classified as high bleeding-risk, 60-65% of patients did not require any transfusion - highlighting the need to better identify these patients as low to intermediate need to requiring transfusion.

Conclusion: This audit demonstrates that fresh blood products are being over ordered when compared to the usage rates. This reflects potential wastage of a scarce resource, increased workload and activity based cost of transfusion, including cross-matching, thawing and dispensation of fresh blood products. These results will form the basis of multi-disciplinary project to develop an algorithm for rational blood ordering which includes standardized Multiplate and ROTEM testing for all major cardiac surgery.

294. Improving cold chain blood supply to theatre

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Aim

To improve cold chain traceability of red cells to theatres at a large tertiary hospital by trialling delivery in shippers with aim to decommission the theatre blood fridge and improve safety and traceability of blood stored outside of Transfusion laboratory

Method

Validated blood shippers and temperature data loggers were issued by the Transfusion laboratory to theatres as a 'point of care blood fridge' during operations where red cells were requested. The laboratory IT system provided traceability of the red cells, barcoded shipper and data logger. A comprehensive education program was developed by Transfusion Medicine for Porters and Theatre staff. The trial was audited against NSQHS Standard 7 criteria 7.7: Ensuring the storage of blood is consistent with best practice. The following data points were collected: Patient details, Theatre number, time issue and return of shipper, average hours in theatre, procedure type, number of units issued and transfused. Non-conformances were raised using the hospital incident management system.

Results: 8 week trial

Shippers	Hours in Theatre	Red cells issued	Red cells transfused
142 (mean 18/week)	Mean = 4	364 (median 2 / shipper)	133 (36%)

Non-conformance: n = 1 Shipper left in theatre after staff vacated

Blood waste: 0 units. All red cells returned within acceptable storage temperature (2-6°C)

Conclusion

We successfully trialled use of "point of care" blood in validated shippers in lieu of the Theatre blood fridge. The education program was effective in ensuring blood shippers were not relocated to wards with patients. Theatre staff reported the shipper were easier to manage than the requirements of documentation and maintenance of a blood fridge. The number of unused shippers arises opportunity to reduce the number of blood requests through education on blood utilisation. This quality improvement activity was endorsed for routine practice at end of trial.

297. Managing the risks of CMV transmission in transplant and other patients at risk

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CMV continues to be one of the most common infections and the most significant viral infection occurring in tissue and organ transplant recipients. Inadequate control results in disease with extended hospital stays, potential graft loss, and increased post-transplantation mortality. Strategies for the prevention of CMV disease after transplantation include i) prevention of acquisition through donor/recipient matching, ii) universal antiviral prophylaxis and iii) pre-emptive strategies, all recommended in International Consensus Guidelines such as Kotton 2013.

Risks of post transplant CMV can be quantitated to some extent, based upon markers including serostatus (higher for R+ HSCT recipients), net state of immunosuppression, GVHD, type of transplant (allogeneic > autologous), standard myeloablation (greater than non myeloablative), ICU admission, and viral factors including higher viral loads and concomitant viral infections such as HHV6. Not all of these risk factors are confirmed in all studies, but they all have some biological plausibility. Prevention strategies for CMV present some difficulties, given the ubiquitous nature of the infection, that seropositive recipients have latent virus present lifelong, that some of the risk factors are unmodifiable (such as development of GVHD), the relatively high rates of infection in the transplant population, and continued acquisition from adults and (particularly) children through intimate contact. Reduction in transmission via transfused blood products can be achieved via i) screening of donors and procedures such as use of CMV blood products from seronegative donors, testing of blood with provision of CMV DNA negative products, use of blood from long term seropositive donors, ii) use of white cell reduced blood components, relying on the cell-associated nature of CMV infection, and iii) specific removal of CMV, currently used in experimental studies. The optimal strategy/s for preventing CMV transmission to recipients have been studied, and reviewed in meta-analysis (Vamvakas 2005), and general reviews (many, but including Ziemann 2013, Ball 2017). The risks present due to CMV infection, and methods for ameliorating them will be discussed.

298. Postnatal Cytomegalovirus (CMV) transmission in VLBW infants: Strategies for prevention

Josephson C

Blood, Tissue, and Apheresis Service

Postnatal CMV infection causes serious morbidity and mortality in VLBW infants. The source of infection has most often been attributed to CMV seropositive blood donors with CMV residing in the monocytes and plasma of those donors. CMV seronegative blood donors were chosen for transfusion to this population and in some areas of the US leukoreduction was added to the CMV seronegative strategy or used alone to prevent transmission. Despite these measures post-natal CMV infections continued to occur. A recent prospective birth cohort study, the largest in the modern era of blood banking, performed in the metro-Atlanta area, over 3 years, was specifically designed to estimate the risk of postnatal CMV transmission from transfusion of CMV-seronegative and leukoreduced blood and maternal breast milk. The results were published in *JAMA Pediatrics* in 2014 revealing that among 539 VLBW infants the cumulative incidence of postnatal CMV infection at 12 weeks was 6.9% (95% CI, 4.2%-9.2%); 5 of 29 infants (17.2%) with postnatal CMV infection developed symptomatic disease or died. Over 2000 transfusions were administered among 57.5% (n = 310) of the infants and none of the CMV infections was linked to transfusion, resulting in a CMV infection incidence of 0.0% (95% CI, 0.0%-0.3%) per unit of CMV-seronegative and leukoreduced blood. Surprisingly, 27/28 infections occurred among infants fed CMV-positive breast milk (12-week incidence, 15.3%; 95%CI, 9.3%-20.2%). The implications of these findings call into question how to prevent CMV transmission through breast milk and what, if any, are the potential long-term neurodevelopmental complications of asymptomatic infection.

299. Changing practice - the POW experience

MacCallum S

Prince of Wales Hospital

The ANZSBT Guidelines for Transfusion and Immunohaematology Laboratory Practice were released in November 2016 and (among other things) recommended restricted indications for the use of CMV-negative products – leucodepleted products should be considered ‘CMV-safe’ for recipients of solid organ transplants, and haemopoietic stem cell transplants as well as all haematology and oncology patients and immunodeficient patients. These guidelines align Australian practice with the UK (SaBTO CMV Position Statement 2012) as well as other international centres.

Randwick Blood Bank has an on-site irradiator and serves a paediatric haematology/oncology service with allogeneic and autologous bone marrow transplant, an adult haematology/oncology service with autologous bone marrow transplant, paediatric and adult renal transplant and the Royal Hospital for Women.

The new Guidelines were greeted with some suspicion across the campus and required time and a variety of strategies to allow their eventual adoption. Guidelines are traditionally focused on what to do, but light on how to do it, and implementation remains a dark art.

Helpful strategies at Randwick will be discussed and included

1. Chair of transfusion committee willing to drive the change
2. Blood bank scientists well educated and aware of the need to change
3. Communicating the scientific basis for change – residual risk estimate of CMV transmission from leucodepleted red cells and less than 1 in 1 million (Seed et al), availability of CMV-PCR testing on site, and the predicted inability of supply of CMV-negative products to keep up with demand.
4. Presenting information in a variety of locations
5. Working to get key clinicians on-side
6. Constantly ascertaining the state of peer practice
7. Emphasis on harmonisation across the paediatric hospitals

The Guidelines will be implemented almost a year to the day they were issued, and will be monitored closely by the Transfusion Committee.

300. Are we there yet - a national position statement for clinical use of CMV seronegative blood products?

Daly J

Abstract not supplied

301. New uses for platelets - lysate and gel

Loh C

Australian Red Cross Blood Service

Platelet components in Australia have a shelf-life of only 5 days. The ability to transform expired platelets that would otherwise be discarded into a new product is attractive. Even after expiry, platelets are a source of useful bioactive substances that promote cell growth, anti-inflammatory and antimicrobial effects. These bioactive substances can be obtained by lysing platelets to make a product known as platelet lysate, which can be used for cell culture or to make a platelet gel that promotes wound healing. Methods for production of these two novel products, platelet lysate and platelet gel, are currently being developed at the Blood Service.

Platelet lysate can be substituted for animal derived serum as a supplement to growth media for the *ex vivo* propagation of therapeutic mesenchymal stromal cells (MSCs), which removes the risk of bovine pathogen transfer and xenoimmunization, thus increasing patient safety. MSCs are increasingly used in many clinical trials due to their immunomodulatory capacity and their ability to differentiate into various cell types, including bone, cartilage, adipose, and neuronal cells.

Platelet gel can be produced by adding clotting agents such as calcium and thrombin to lysed platelets. Soluble fibrinogen in the platelet lysate is converted into insoluble fibrin strands, producing a gel like material that can be applied topically to enhance wound healing in patients with ulcers and skin infections. Currently, autologous platelet gels are typically used. However, for patients such as those with haematological disorders and the elderly, donation of sufficient autologous blood is not possible, and an allogeneic platelet gel product would be beneficial for these patients.

In conclusion, the uses of human platelet derived products are gaining increasing attention especially in the field of cellular therapy. The development and characterisation of platelet lysate and platelet gel will be presented.

302. Managing oxygen during storage improves red blood cell quality

Dunham A

New Health Sciences

Annually, approximately 90 million red blood cell transfusions are given to some of the sickest patients around the world. Efforts to improve the quality of the blood supply have historically focused on improvements in additive solutions, pathogen testing and ensuring a ready inventory of compatible, affordable products for local health care systems. Attempts to understand how the quality of red blood cells impacts patient outcomes have relied on randomized clinical trials which use the age of blood as a surrogate for the quality of the red blood cells. These studies lean heavily on assumptions that the changes occurring during storage affect all red cells similarly and that different patient groups will respond to the changes in red blood cells in a similar manner. However flawed these assumptions are, the studies have shown that fresh blood does not offer an advantage to aged blood. The converse has not been shown, that aged blood is safe, and extensive research continues to understand whether the changes occurring to blood during storage reduces the efficacy and safety of the resulting transfusion.

Oxidative damage has been invoked in much of the research on blood quality as an important source of the red cell storage lesion. Demonstrating the importance of oxidative damage on transfusions requires:

- 1) the presence of an O₂ dose dependence of red cell damage
- 2) the dose of O₂ in current red cell components is uncontrolled and widely variable
- 3) a preclinical evaluation of quality improvement due to reducing oxidative damage
- 4) data showing clinical efficacy and safety of red blood cells with reduced oxidative damage

While clinical evaluations of safety and efficacy are not complete, our results to date show the damage to red blood cells during storage is O₂ dose dependent, the levels of O₂ in donated red blood cells is highly variable and uncontrolled, and third, controlling O₂ during storage has a significant effect on the safety and efficacy of red blood cells used for resuscitation in a preclinical haemorrhagic shock model. This research supports the continued development of an oxygen-managed red cell product that can be integrated into current red cell manufacturing, offering the potential for improvements in stored red cell quality.

303. New Indications for IVIG; New proteins from plasma; and, disruptive technologies

Maher D

CSL Behring

Intravenous immunoglobulin has been the driver for plasma collection in western countries for many years and in Australia, demand for immunoglobulins (IVIG and SCIG) continues to grow at over 10% per year. Access is funded by the National Blood Authority in Australia largely based on a document authored by clinical experts titled 'Criteria for the Clinical Use of Intravenous Immunoglobulin in Australia'¹. Clinical evidence for many of the funded indications is limited. CSL Behring has been contributing to the body of evidence through sponsored clinical trials, the most recent being the 'PATH' study of Hizentra, a subcutaneous Ig product, in Chronic Inflammatory Demyelinating Polyneuropathy.

Efforts to identify new therapeutic proteins from plasma continue with a particularly interesting and exciting prospect being Reconstituted HDL, made from plasma derived apolipoprotein A. CSL 112 has been shown to facilitate reverse cholesterol transport based on biomarkers measured in Phase II studies. CSL Behring is preparing to commence a large Phase III trial of CSL 112 in patients with Acute Coronary Syndrome (ACS). If this trial shows a clinically meaningful reduction in the risk of second events after ACS, we could see a dramatic change in the drivers for plasma collection in the future.

Finally, recombinant Fc molecules show promise as an alternative to high dose IVIG in the treatment of autoimmune disorders. Such therapies have the potential to 'disrupt' the plasma fractionation industry by reducing demand for IVIG. CSL has a research stage program investigating various forms of recombinant Fc molecules and in collaboration with Momenta Pharmaceuticals has a candidate molecule in development expected to enter clinical trials in the near future.

<https://www.blood.gov.au/ivig-criteria>

304. A pilot study on the fate of cytomegalovirus-seronegative blood products at 3 tertiary-referral hospitals

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Aim

Supply of cytomegalovirus (CMV) seronegative products in Australia is an ongoing challenge. Australian trends in demand for blood products derived from CMV-seronegative donors indicate insufficiency of supply around 2017/2018. Clinical data evaluating how these products are currently being utilised is limited. Therefore this pilot study aimed to identify the fate of CMV-negative blood products and indications for use.

Method

All CMV-negative red blood cell (RBC) and platelet components issued to three New South Wales based tertiary hospitals in May 2016 were identified (n =1219). The reason for selection of CMV-negative blood products were evaluated using an electronic survey form. Data was gathered on the reason for discard, selection of the product based on the recipients CMV-IgG status, indication for CMV-negative requirement or selection based on inventory requirements.

Result

Transfusion data was collected on 411 units. Of these, 127 (30.9%) were transfused to CMV-IgG positive recipients. 67 (16.3%) were transfused to CMV-negative requiring recipients (28 transplantation, 26 lymphoma/leukaemia, 6 obstetric/neonatal, 7 other), 144 (35%) CMV-negative units were selected for transfusion based on their irradiated status, 137 (33.3%) were transfused as they were close to expiry, 28 (6.8%) were chosen for specific phenotype requirements with the remaining 35 (8.6%) units transfused for unknown reasons. Of the 1044 RBC and 175 platelet CMV-negative components issued, 32 (2.6%) were discarded by the institutes.

Conclusion

The majority of CMV-negative blood products are not used for CMV-negative requiring recipients. Alteration to inventory management appears as an imperative to ensure continued supply for CMV-negative requiring recipients.

305. Red blood cells: the immune system's hidden regulator.

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Evidence is mounting that the most abundant cell type in the body, red blood cells (RBCs), are more complex than previously understood. Studies have reported that RBCs from healthy individuals regulate immune cell activity¹, but stored RBCs or RBCs from inflammatory cohorts are dysfunctional^{2,3}. Those studies did not, however, determine the mode of action that enables RBCs to affect immune cell function. Our group determined that RBCs are the primary reservoir for over 30 cytokines, chemokines, and growth factors in blood. This study aimed to explore the implications of RBC-associated cytokines on immune cell function. RBCs were isolated from healthy volunteers, and cytokine quantification of RBC lysates and secreted cytokines was achieved using Luminex® technology. Exposure to an immortalised cancer cell line in culture was used to alter the cytokine profile of intact RBCs and the effect of these 'primed' RBCs on T cells was evaluated using flow cytometry.

Following cell line exposure, primed RBCs were loaded with significant concentrations of cancer-associated cytokines. Then, in the presence of these primed RBCs, T cells (Jurkat cells and peripheral blood mononucleated cells) proliferated significantly more than naïve RBC stimulated controls, were no longer protected from stimulant-driven activation, and secreted a variety of cancer-related cytokines. This study supports the hypothesis that RBCs act as a dynamic buffer for cytokines in blood through binding and release, and that RBCs affect the activity of immune cells. Cell-cell interactions alter RBC cytokine profiles and modulate their effect on immune cells. These findings may have implications in the study of the storage lesion and the inflammatory processes that can occur following RBC transfusion.

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306. Maximising inventory potential of platelets by delaying cold-storage

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Aim

Cold-stored platelets are being assessed as an alternative to conventional room temperature storage, due to their increased haemostatic potential and reduced risk of bacterial growth. However, cold-stored platelets are cleared more rapidly from circulation, which may reduce their suitability for prophylactic transfusions. In an effort to maximise their utility, platelets could be stored conventionally until near expiry (4 days) for prophylactic transfusions, then refrigerated for use in bleeding patients. As such, the aim of this study was to compare the quality of platelets stored under refrigerated conditions and platelets stored conventionally until near expiry followed by refrigeration.

Methods

Two ABO-matched buffy coat-derived platelets were pooled and split to form matched pairs (n=8 pairs). One unit was immediately stored at 2-6 °C (day 1 post-collection; cold) while the second unit was stored at 20-24 °C with constant agitation until day 4 then stored at 2-6 °C thereafter (cold-delayed). All units were sampled over an extended 21 shelf-life. Data were analysed using two-way repeated measures ANOVA.

Results

During storage, cold and cold-delayed platelets maintained a similar platelet count. While pH and HSR were significantly higher in cold-delayed platelets, other metabolic markers including lactate and glucose concentration did not differ significantly. Further, surface expression of the activation marker CD62P, phosphatidylserine exposure, release of soluble CD62P and microparticles did not differ significantly, suggesting similar activation profiles. Aggregation responses of cold-delayed followed the same trend as cold platelets once transferred to cold-storage, gradually reducing over the storage period.

Conclusion

The metabolic and activation profile of cold-delayed platelets was similar to cold stored platelets during a 21 day storage period. These data suggest that transferring platelets that are near expiry into refrigerated storage may be viable for maximising platelet inventories, by extending the shelf life of platelets beyond 5 days.

	Cold (Day 7)	Cold-Delayed (Day 7)
pH	7.01 ± 0.05*	7.18 ± 0.07*
HSR (%)	26 ± 5	42 ± 8*
CD62P (MFI)	90 ± 17	93 ± 21

*indicates p<0.05

307. The risk of anti-Yt^a causing acute haemolytic transfusion reaction

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Aim

Yt^a is a high frequency antigen (99.7%) and given the low incidence of Yt^a negative people (0.3%) providing blood to patients with preformed anti-Yt^a is logistically problematic. This study aims to assess the incidence and severity of acute haemolytic transfusion reactions (AHTR) to Yt^a positive donor red cells in recipients with preformed anti-Yt^a antibodies.

Method

52 patients with anti-Yt^a were identified by the Red Cell Reference Laboratories of Australian Red Cross Blood Service over the last 20 years. Their transfusion records were reviewed for evidence of AHTR.

Results

Thirteen patients were confirmed to have received a red cell transfusion. Ten patients received allogeneic red cells while 1 had autologous red cells and 2 received frozen Yt^a negative units. None of the patients who received allogeneic red cells had documented acute haemolytic reactions despite receiving incompatible (likely Yt^a positive units). Antibody titre when available, did not seem to affect outcome of transfusion.

Conclusion

This is the largest retrospective study to date of patients with anti-Yt^a as there has been limited data regarding the haemolytic potential of this antibody through several published case reports. Our data and most data from the literature fails to demonstrate significant haemolysis when transfusing Yt^a positive donor red cells to a Yt^a negative recipient with pre-formed anti-Yt^a antibodies. For this reason, we suggest that all other considerations for safe transfusion, such as other potentially significant antibodies and patient specific requirements (e.g. irradiated, cytomegalovirus negative) are prioritized above provision of Yt^a negative units.

308. Refrigeration of platelets affects the release of biological response modifiers (BRMs), promoting a procoagulant milieu

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Aim: Conventional storage (20-24°C) of platelets results in progressive activation, including the release of biological response modifiers (BRMs), which may ultimately affect component function upon transfusion. Refrigerated storage (2-6 °C) is attractive as it may extend platelet shelf-life and improve their haemostatic capacity. However, the mechanisms mediating this effect are yet to be fully elucidated. As such, the aim of this study was to define the profile of BRMs present in the supernatant of refrigerated platelets.

Methods: Buffy coat-derived platelets were pooled, split and stored at either room temperature (RT) or under refrigerated (cold) conditions (n=8 in each group) for an extended storage period of 21 days. The supernatant fraction was isolated throughout storage by centrifugation. Microparticles were enumerated by flow cytometry. The functional capacity of the supernatant was assessed using a phospholipid-dependant FXa-based clotting assay (STA-procoag-PPL) and calibrated automated thrombogram (CAT). Coagulation factors and cytokines were assessed using a coagulation analyser and ELISA, respectively. Data were analysed using two-way ANOVA, where a p-value of <0.05 was considered statistically significant (*).

Results: A significant increase in the number of platelet microparticles was seen in cold platelets. The supernatant of cold platelets induced PPL-clotting two-fold faster than RT platelets. Similarly, platelet supernatants from cold units generated more thrombin (peak) at a faster rate (lag-time) than RT-controls. Procoagulant clotting factors (FV, FVIII, FIX) decreased over storage at a similar rate in RT and cold units, whilst the anticoagulant Protein S was better preserved in cold platelets. The alpha granules were retained better within cold platelets, as evidenced by reduced concentration of archetypal cytokines (CD62P, PF4, RANTES) in the supernatant.

Parameter	Pool Day 1	RT Day 7	Cold Day 7
Microparticles (x10 ⁶ /L)	1489±965	3357±1744	13208±6088*
PPL-clotting time (sec)	68±4	65±4	35±3
CAT peak (nM)	46±8	60±5	80±6*
CAT lag-time (min)	8±1	7±1	6±0*
RANTES (ng/mL)	26±9	140±17	39±7*

Conclusion: Refrigeration results in significant alterations to the composition of the releasate of platelet concentrates. The differences in this mixture of compounds may promote the improved haemostatic response typified by cold-stored platelets.

Key words: Platelets, refrigeration, microparticles, cytokines.

Conflict of interest: The authors have no conflict of interest to disclose.

309. A risk tool to mitigate severe haemolytic reactions due to high isoagglutinins in apheresis platelets

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Background

Group O apheresis platelets are labelled “low Anti-A/B” if IgG tests < 1:250 and IgM < 1:50. There remains a lack of national guidelines to help stratify the risk of severe haemolytic transfusion reactions when minor ABO mismatched apheresis platelets are the only product available for transfusion.

Aims

To develop a pathology service Risk Matrix Tool to assess the risk of transfusing a minor ABO mismatched Group O apheresis platelet based on ABO haemagglutinin titres to improve patient safety.

Methods

A comprehensive literature review to determine the incidence, and the risk of haemolysis, due to isoagglutinins was performed to guide the development of a Risk Matrix Tool (see below).

Results

There is a lack of international standardisation of Anti-A/B titre methodology whilst ABO titres appeared to have variable potential for haemolysis. There appeared to be multiple donor and recipient factors contributing to the severity and/or likelihood of reactions. However, an expert panel developed a conservative Risk Matrix Tool to assist clinical decision-making when minor ABO mismatch platelets must be transfused.

Risk Matrix Tool

IgM Titre (Saline)	IgG Titre (IAT)	Risk
<256	<512	Low-Moderate
512-1024	512-1024	Moderate
1024-2048	1024-2048	High
>2048	>2048	High - Extreme

Conclusions

ABO haemagglutinin reactions are complex, multifactorial and likely under-reported. If forced to cross groups the Risk Matrix informs the clinical decision to transfuse, however, the Blood Service must provide Anti A/B titres, or the pathology transfusion service be able to perform Anti A/B titres. Furthermore, once the titre is known and a decision to proceed with the transfusion is made, cautious administration of the product such as plasma reduction or washing, or infusing small volumes of the product whilst the patient is closely monitored by experienced staff could further improve patient safety.

Whilst there is still lack of universal ABO haemagglutinin titre methodology and no nationally endorsed guidelines defining risk, transfusion scientists and clinicians are unable to protect patients from potentially catastrophic transfusion reactions.

310. Clinical practice improvement tools play a part in blood management for maternity

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Aim: Anaemia is the second most common medical condition in pregnancy in the state.¹ Antenatal iron deficiency (ID) and/or anaemia (IDA) is associated with an increased use of red cell transfusion postpartum. Our 2015 hospital data shows that anaemic maternity patients at delivery (Hb \leq 110 g/L) had six times higher chances of being transfused compared to non-anaemic patients. This clinical practice improvement (CPI) aimed to increase use of oral iron therapy in women with iron deficiency to reduce the rate of anaemia at delivery.

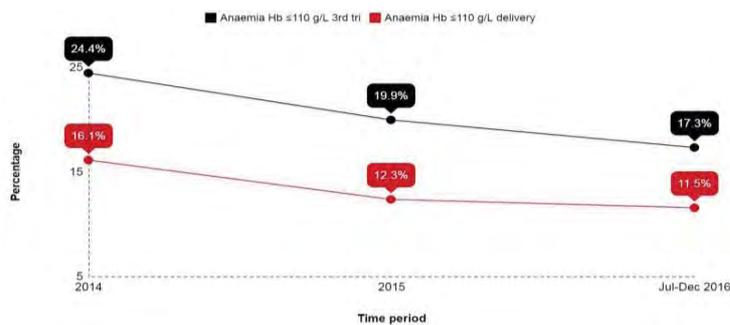
Methods: CPI methodology was used to determine interventions and data monitoring. The key interventions were:

- The adaptation of obstetric anaemia assessment and management flowcharts and patient information on oral iron
1. Maternity staff education on using CPI tools from November 2016 to January 2017
 2. A telephone audit of 30 women diagnosed with iron deficiency to assess diagnosis awareness and the utility of the patient information handout.

Key data collected and correlated with interventions were: patient haemoglobin and ferritin levels, red cell transfusions, staff feedback and patient feedback.

Results: The rate of anaemia decreased at 3rd trimester and delivery (see Figure 1). Haemoglobin and ferritin requests increased in each trimester post-implementation. Ferritin results showed 67% were iron deficient (\leq 30 μ g/L). The CPI tools provided a 'long needed guidance and consistent approach' according to maternity staff with majority endorsing the tools. The telephone audit revealed 83% of women were informed of the iron deficiency and 72% commenced oral iron supplementation. There was a decrease in the numbers of monthly red cell transfusions and specifically 2 or more red cell units per transfusion episode.

Figure 1. Rate of anaemia at 3rd trimester and at delivery



Conclusion: CPI tools provided positive practice improvements in maternity blood management; namely, increased detection of ID/IDA, increase use of oral iron therapy, decreased rate of IDA intrapartum, and decreased red cell transfusions.

No conflicts of interest to disclose.

¹ Pregnancy outcome, 2014.

311. Are we doing justice to the 2nd pillar of patient blood management—addressing iatrogenic anaemia?

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Background: Patient blood management (PBM) includes a key focus on conserving the patient's own blood to reduce the need for transfusion and its associated risks, improving patient outcomes. The second pillar is "minimisation of blood loss" which is targeted by the National Blood Authority PBM guidelines to address iatrogenic anaemia.

Aims

- To assess if policies exist to minimise phlebotomy in four Australian jurisdictions
- To identify practices supporting minimal blood sampling and to identify barriers

Methods: Health services (n=149) were invited to complete an online audit, from January to February 2017, regarding local policies and practice to reduce iatrogenic blood loss.

Results: Seventy-eight (52%) health services responded. Six reported a policy specifically supporting minimal blood sampling to reduce iatrogenic anaemia, while 66 (85%) practiced minimal blood sampling to some degree; however, often for other reasons e.g. for convenience, time efficiencies or patient comfort, as shown in table 1. Commonly this occurred in paediatrics, intensive care and difficult to bleed patients.

Table 1. Strategies reported in health services.

Strategy	Number of health services* reported		
	reduce anaemia	iatrogenic	other reasons
Point-of-care testing	4 (5%)		41 (53%)
Small volume phlebotomy tubes	13 (17%)		23 (29%)
Bundled scheduling of blood sampling	10 (13%)		23 (29%)
Frequent evaluation of routine orders	9 (12%)		18 (23%)
Non-invasive monitoring	6 (8%)		18 (23%)
Closed system sampling	10 (13%)		9 (12%)
Charting of cumulative phlebotomy loss	1 (1%)		1 (1%)

*A health service could select more than one practice.

Potentially unnecessary blood sampling practices were reported: ordering specified test sets (n=30) and routine orders (n=19) without considering the individual. Explanation for a lack of policy supporting minimal sampling was commonly (n=38) due to "practice not considered".

Conclusions: Components of Pillar 2 are being overlooked. Participating health services did not have policies supporting minimal blood sampling as a PBM approach and where strategies are used, this is for reasons other than prevention of iatrogenic anaemia. Recommendations include: increasing awareness of iatrogenic anaemia, and development of a cumulative phlebotomy loss tool to highlight need for practice change.

312. Learning across borders: Improving transfusion practice in the developing world through online learning

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Background

BloodSafe eLearning Australia (BEA) (www.bloodsafelearning.org.au) provides knowledge of patient blood management and clinical transfusion practice. The program has more than 425,000 registered learners who have completed more than 850,000 courses. While the program is aimed at, and promoted to, Australian healthcare workers, all courses are freely available on the internet.

Aim

To analyse the uptake, usage and feedback on BEA courses by international learners and to determine changes required for an international audience, particularly in the developing world.

Method

A retrospective analysis of learner registration data, course completion records and course evaluation questionnaires to investigate the uptake, usage and feedback by international users.

Results

Analysis of registered international learners shows that as of 30 June 2017 the BloodSafe eLearning program had 8,309 registered learners from outside of Australia, who had completed 12,095 courses.

These learners come from 178 countries - 62.7% from very high HDI countries, 13.8% high HDI countries, 18.1% medium HDI countries and 4.2% from low HDI countries.

Analysis of learners' profession showed that 57.9% were nurses or midwives and 24.2% were medical with the remainder from a range of other health areas.

Analysis of learner evaluation questionnaires showed that 93.5% of respondents believe that the course/s provided them with increased knowledge and 63.6% can make changes to their clinical practice based on this knowledge. Free text comments showed that the courses are of high quality, relevant and up-to-date. Challenges identified by users included the need for English language literacy, a greater use of a glossary, and localisation of terminology.

Conclusion

This analysis demonstrates that elearning can provide consistent, credible and reliable education on transfusion medicine knowledge, practice and governance to healthcare professionals anywhere in the world and provides an opportunity to improve transfusion medicine on a large scale in a very cost-effective manner.

313. Screening for iron deficiency in pregnancy with serum ferritin: A patient blood management strategy

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Aim

Although current guidelines recommend that screening for anaemia in pregnancy should include haemoglobin estimation only, iron depletion in pregnancy may be missed. We aimed to determine the value of screening for iron deficiency with serum ferritin in pregnancy.

Method

A process of universal ferritin screening was adopted, with iron replacement given for deficiency (ferritin < 30 µg/L) during a patient blood management intervention at Canberra Hospital beginning in 2015. Haemoglobin and ferritin readings during pregnancy were retrospectively analysed before and during the intervention. Third trimester haemoglobins of women with low or normal ferritin levels in first trimester before and after the intervention were compared. Results were compared with 2 tailed T tests.

Results

Prior to the intervention, 17 (32.9%) of 70 women with ferritin levels determined in first trimester were iron deficient, and they had lower haemoglobin levels in third trimester compared with non-iron deficient women (121.3 v 128.7g/L, P=0.02). During the intervention 31 (29%) of 107 women screened in first trimester were iron deficient, but there was no significant difference in the haemoglobin readings in third trimester between the iron deplete and iron replete women (123.9g/L v 125.9g/L, P=0.4), due to a program of iron replacement. None of the iron deficient women in first trimester were anaemic.

Conclusion

Iron deficiency is common in pregnancy. Screening with serum ferritin rather than haemoglobin alone more reliably detects iron deficiency, enabling early intervention that results in improved haemoglobin levels prior to delivery.

314. It's time to STOP - intervention to improve compliance with transfusion documentation

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Aim: To improve compliance with documentation of completion time and observations related to red blood cell (RBC) transfusion.

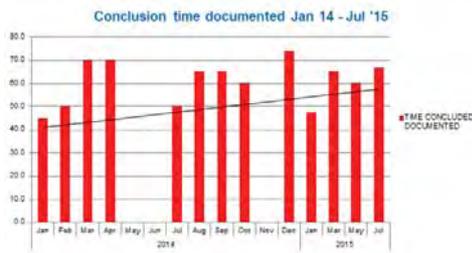
Method: The SVHM transfusion team audit the documentation of 30 randomly selected RBC transfusions every 2 months. The SVHM transfusion committee (TC) noted significant non-compliance with documentation of transfusion completion time and required patient observations (temperature, pulse, respiration rate, blood pressure and oxygen saturation at baseline, 15 minutes into the transfusion, and at transfusion completion).

The intervention proposed by the TC involved attaching a cardboard 'luggage' tag (known as a 'STOP' tag) to each RBC unit leaving the transfusion laboratory. The 'STOP' tag (Picture 1) was intended to prompt clinicians to complete required documentation at the conclusion of each RBC transfusion. The tags were trialed for 3 months from August to October 2015, with compliance monitored as usual during this period. Staff were notified of the intervention via the Senior Nurse Advisory Council (SNAC) and the quarterly Transfusion Bulletin.

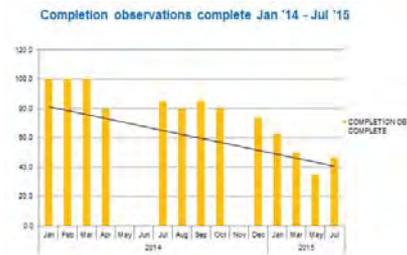
The intervention initially appeared to improve compliance, but this declined towards the end of the trial period. The TC decided to reinstate the 'STOP' tags for 1 month every quarter with ongoing audit of compliance. 'STOP' tags were in use in June and September 2016, as indicated by the arrows in Graphs 3 and 4.

Results

Graphs 1 and 2: compliance preceding the intervention.



Graph 1



Graph 2



Picture 1: 'STOP' tag

Graphs 3 and 4: compliance after the intervention.



Graph 3



Graph 4

Conclusion: The ongoing use of 'STOP' tag reminders has led to an overall increase in compliance with documentation of transfusion completion time and observations, though this varies from month to month. 'STOP' tags will be in use for one month each quarter going forward in an attempt to increase compliance.

No conflict of interest to disclose.

318. Ironing out the emergency department - progress on identification and management of iron deficiency anaemia

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Aim

To compare progress on anaemia management and transfusion practice in the emergency department (ED) of a large academic hospital as part of a comprehensive audit program in place for compliance with NSQHS Standard 7 and national patient blood management guidelines.

Method

Audit of patient presentations with anaemia to the ED in May 2017 and comparison with results from July 2015. Patients were selected from Monash Health blood bank records indicating transfusion of red blood cells (RBC). Transfusion was categorised as “appropriate”, “appropriate indication (not quantity)” or “possibly not required” based on clinical and laboratory records. Alignment with single unit policy was assessed based on clinical information or haemoglobin measurement following one RBC in appropriate cases. Iron status was categorised as “deficient”, “probable”, “unlikely” or “uncertain” using FBE, blood film and iron studies.

Results

Of 33 cases presenting in May 2017 (14 with serious bleeding, 19 other bleeding), transfusion was adjudicated as clinically appropriate in 91% but not in quantity for 21%, whilst 9% were possibly not clinically indicated. This was compared with 27 cases in 2015 of which 92% transfusions were appropriate but not in quantity for 62%, whilst 12% were possibly not required.

Single unit policy was met in 50% of cases in 2017 compared with 61% in 2015.

In 2017, 64% of patients had iron studies performed, up from 59%. Of 11 patients with probable or definite iron deficiency in 2017, six received iron infusions, two had oral replacement (total 73% receiving iron) and three were not documented to receive replacement; compared with 7 of 16 patients (44%) with probable or definite iron deficiency documented as receiving iron replacement in 2015.

Conclusion

We are making progress on identification and management of iron deficiency in ED patients presenting with anaemia. However, there is still room for improvement to avoid unnecessary RBC transfusion.

No conflict of interest to disclose

319. Novel platelet storage modalities: Combining pathogen inactivation treatment and cold-storage

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Aim

Cold-storage (cold) and pathogen inactivation (PI) may facilitate an extension of the platelet shelf-life, compared to conventional room-temperature (RT) platelet storage. However, both treatments are purported to effect different aspects of platelet quality. The aim of this study was to determine whether combining PI and cold-storage (cold-PI) may balance out the deleterious effects induced by each method individually, resulting in a better quality, longer lasting platelet product.

Method

A pool and split design was used to generate four study arms: RT, cold, RT-PI and cold-PI. On day 1, platelets were left untreated or PI-treated using the THERAFLEX UV-Platelets System (MacoPharma). One unit from each pair was then stored at RT (20-24°C) or refrigerated (2-6°C). *In vitro* quality and function were tested over 9 days using flow cytometry and aggregometry. Data was analysed using two-way ANOVA where $p < 0.05$ was considered significant.

Results

Cold-stored platelets demonstrated an enhanced agonist-induced aggregation response compared to RT-stored platelets, and PI-treatment had no additional effect. Surface P-selectin expression was elevated in cold-stored platelets, and no further increase was seen in PI-treated groups ($p < 0.0001$). Binding of PAC-1 to the activated conformation of GPIIb/IIIa was increased by both cold and PI-treatment individually, and the cold-PI platelets demonstrated the highest PAC-1 binding ($p = 0.0011$). Similarly, phosphatidylserine externalisation was increased by PI-treatment from day 5 of storage, with the cold-PI platelets showing the highest annexin-V binding of the four treatment groups ($p < 0.0001$).

Conclusion

PI treatment and subsequent storage under refrigerated conditions differentially affects aspects of platelet activation. However, platelet aggregation, as an indicator of function, was enhanced compared to conventionally stored platelets. Further data is required to determine whether combining these storage techniques would facilitate an extended shelf-life.

Conflict of interest

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

320. Relationship between HbS level pre and post erythrocytapheresis in children with sickle cell disease

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Background: Red cell exchange transfusion (RCE) has been shown to be an effective therapy in sickle cell disease (SCD). There are no randomised controlled trials to suggest the optimal target HbS level pre- or post-RCE. A recommendation of moderate strength (2014), suggests HbS levels be maintained less than 30% prior to the next transfusion in paediatric patients on regular transfusion therapy.(1) A recent abstract (American Society for Apheresis 2017) suggested a target post-RCE HbS of $\leq 10\%$ was likely to predict maintenance of HbS levels within this range(2).

Aim: To establish the relationship between post-RCE HbS% and HbS% prior to the next RCE in a cohort of children on regular apheresis at The Royal Children's Hospital (RCH). To determine if a target post-RCE HbS level of $\leq 10\%$ can be used to predict a HbS of $< 30\%$ prior to the next RCE.

Method: A retrospective audit of laboratory records was conducted for patients with SCD undergoing regular RCE at RCH from January 2015 to July 2017. Data on frequency of RCE, and pre- and post-RCE HbS% were collected. RCE episodes occurring < 20 or > 45 days apart were excluded.

Results: Six patients underwent regular RCE during the study period, with a total of 95 RCE episodes meeting the inclusion criteria. There was a correlation between the post-RCE HbS% and the HbS% prior to the next RCE, $R=0.81$. A post-RCE HbS of $\leq 10\%$ was achieved in only six (6.3%) of RCE episodes, of which one had HbS $< 30\%$ prior to the next RCE.

Table 1: Frequency of pre-RCE HbS $< 30\%$ according to post-RCE HbS category.

Post-RCE HbS (%)	RCE episodes achieving this post-RCE HbS Number (%)	HbS $< 30\%$ prior to next RCE Number (%)
$\leq 10\%$	6 (6.3)	1 (16.7)
10.1 – 15%	20 (21.0)	4 (20.0)
15.1-20%	18 (18.9)	0 (0)
20.1-25%	31 (32.6)	0 (0)
25.1-30%	11 (11.6)	0 (0)
$> 30\%$	9 (9.5)	0 (0)

Figure 1: Relationship between post-RCE HbS and HbS prior to the next RCE.

Conclusion

Good correlation was observed between post-RCE HbS% and the HbS% level prior to the next RCE. Post-RCE levels $< 10\%$ were an infrequent occurrence, therefore it is difficult to establish whether this suggested target should be adopted when ordering RCE in our cohort.

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321. The national rollout of BloodSTAR - Blood system for tracking authorisations and review

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BloodSTAR was developed by the National Blood Authority (NBA) and first implemented in July 2016 as one of the government measures under the National Immunoglobulin (Ig) Governance Program to improve the governance and management of government funded Ig. The aim was to ensure product use and management reflects appropriate clinical practice and represents efficient, effective and ethical expenditure of government funds.

The key benefits of BloodSTAR include;

- ensuring consistency of processes and application of the *Criteria for the Clinical Use of Intravenous Immunoglobulin (IVIg) in Australia (Criteria)*;
- transparency of information enabling support for patients across multiple sites and;
- further development of the Criteria to ensure Ig is directed to where there is evidence of greatest benefit.

BloodSTAR was rolled out across jurisdictions via a staged process. The NBA worked closely with each jurisdiction and relevant health sector stakeholders for more than 12 months to map out key business process changes and to communicate them widely. The NBA in collaboration with the Blood Service managed the transition from the predominantly paper based processes to the online system. Consent to record personal and sensitive information in BloodSTAR was obtained from patients who were authorised to receive Ig product beyond their jurisdiction 'go live' date. Relationships between treating, administering and dispensing facilities were collected so they could be entered to ensure continuity of care and a smooth transition. The NBA delivered a series of webinars and 179 face to face education and training sessions to key users at 55 hospitals/sites.

BloodSTAR is now live in all States and Territories except NSW and manages all patients who are authorised under the Criteria to receive government funded Ig. There are currently over 7700 patients with active authorisations and 6800 users accessing the system as either, Authorisers, Medical Officers, Nurses, Admin Support Officers or Facility Administrators.

322. Targeted exon sequence based genotyping to resolve cases with problematic Rh typing

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Background

The clinically significant Rh blood group system is extremely polymorphic. Some polymorphisms arise from an array of single nucleotide variants on the *RHD* or homologous *RHCE* gene. Such variants are associated with partial and/or weakened expression of RhD, C/c or E/e antigens. It is difficult to resolve the Rh phenotype(s) by serology and SNP-array typing for some such variants. This study investigates sequence based genotyping to solve such complex cases.

Method

Thirty samples were referred for investigation using TruSight™ One Sequencing Panel and the MiSeq® platform. Read mapping and variant calling were performed using CLC genomics workbench. Phenotyping predictions were made with reference to public blood group variant data bases, primarily the ISBT database.

Result

The average sequencing depth for *RHD* and *RHCE* was 58 and 79. In three cases novel alleles were identified. The first, presenting with weak RhD antigen, carried a c.270G>C in exon 2 of *RHD*, predicting a p.W90C change. The second, presenting with weak E antigen, carried the c.697C>G on the E allele, predicting a p.Q233E change. The third, an antenatal case in a mother of African background and involving maternal alloimmunisation, revealed c.486C>G on the *CE* allele in the father and baby, predicting a p.N162K change in the third extracellular domain of the RhCE protein.

A further case presented with weak RhE antigen attributed to the *RHCE*cE.01* allele (*E^w* phenotype). The remainder of cases resolved as diverse but known variants associated with weak/partial RhD phenotypes (53%), or with unremarkable *RHD* and *RHCE* sequences.

Conclusion

A novel variant in the antenatal case we propose represents a new antigen in the Rh system. Other cases were resolved by identification of both novel and known variants associated with weak antigen expression. Sequence based genotyping is instructive in discovery mode, defining novel RH alleles within the diverse Australian population.

No conflict of interest to disclose

323. Cold-storage of apheresis platelets in plasma is a suitable alternative to conventional storage

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Aim: Cold-storage of platelets increases shelf-life beyond five days by decreasing platelet metabolism and potential bacterial growth. Since there are few recent studies showing the effect of extended cold-storage on platelet quality, the aim of this study was to assess the metabolic and functional aspects of cold-stored apheresis platelets stored in plasma over nine days.

Method: Double apheresis platelet units in plasma (n=6) were pooled and split and stored at either room temperature (RT; 20-24 °C) or refrigerated (2-6 °C). Day 9 results are reported as a prospective expiration target. Units were assessed for metabolism (glucose and lactate) and functionality (aggregation and thrombin generation). Expression of activation markers (P-selectin and phosphatidylserine) and microparticle release were assessed using flow cytometry. A phosphatidylserine-dependant FXa-based clotting assay (PPL) was performed as a measure of procoagulant activity. Data were analysed using two-way ANOVA where a p-value of <0.05 was considered statistically significant (*).

Result: By day 9, the platelet count in cold-stored units was significantly lower than RT platelets, although no aggregates were observed. Compared with RT, cold-stored platelets consumed less glucose and produced less lactate, demonstrating a reduction in the metabolic rate. Platelet aggregation and thrombin generating potential were similar in both groups. Cold-stored platelets were not more activated than RT counterparts, but contained significantly more microparticles, which correlated with a reduction in PPL clotting time.

Conclusion: Extended cold-storage of apheresis platelets in plasma reduced the metabolic rate whilst maintaining platelet functionality, suggesting it may represent a suitable storage alternative for up to nine days.

Parameter tested	Day 1	Day 9	
	Pooled	RT	Cold
Platelet count (x10 ⁹ /L)	1360 ± 197	1385 ± 218	1245 ± 248*
Metabolism			
Glucose (mmol/L)	20 ± 2	10 ± 3	14 ± 4*
Lactate (mmol/L)	5 ± 1	25 ± 3	17 ± 3*
Functionality			
ADP aggregation (% max)	70 ± 11	29 ± 13	37 ± 9
Endogenous thrombin potential (nM*min) [§]	1167 ± 214	2716 ± 856	2227 ± 754
Microparticles			
CD61+/Annexin-V+ (x10 ⁶ /unit) [§]	2628 ± 1054	2264 ± 855	7433 ± 3221*
PPL clotting time (sec)	47 ± 6	31 ± 5	24 ± 3*

Values shown as mean ± SD; *p<0.05 using two-way ANOVA; [§]n=5

325. Pre-op haemoglobin optimisation algorithm and its effects on transfusion rates in elective orthopaedic surgical patients

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Aim: To improve the care and reduce the rate of transfusion of elective orthopaedic patients by reducing the risk of attending with pre-existing anaemia.

Method: An algorithm was developed for patients attending pre-admission clinic for major elective orthopaedic surgery which described the investigation and management of haemoglobin and iron levels.

Haematology, anaesthetics and gastroenterology were involved in the development of the algorithm to ensure appropriate follow up and testing of patients.

Data was collected to assess the use and effectiveness of the algorithm and compared with historical data.

Results

Orthopaedics	2012 N=231 (%)	2015 N=184 (%)	2016 N=205 (%)	2017 N=22 (%)
No. patients anaemic	21 (9)	26 (14)	16 (8)	2 (9)
No. patients with low ferritin	NA	6 -29 tested (21)	27 -194 tested (14)	5 -22 tested (23)
Total no. patients with either	21	27	38	6
No. patients who received treatment	NA	9 -8 received Fe infusion	22 -19 received Fe infusion	3 -3 received Fe infusion
Use of tranexamic acid	NA	78 (43)	131 (64)	14 (64)
Transfusion rate	80 (35)	32 (17)	24 (12)	4 (18)
Single unit transfusions	12/80 (15)	7/32 (22)	8/24 (33)	1 /4 (25)

Transfusion rate in 2012, when the algorithm was first introduced, was similar to audit data from 2009 when the rate was 33%.

At the end of 2015 routine ferritin testing was introduced to determine if we were missing iron deficient patients who were not yet anaemic, previously only anaemic patients had a ferritin added. Routine testing of ferritin continues.

Conclusion: Transfusion rate has decreased over the length of the project but cannot be attributed to the algorithm alone. Over the same time period there has been an increase in tranexamic acid use and single unit transfusions. This algorithm will be used with other surgical groups.

326. RhD - what's the problem?

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Aim

Describe incidents related to RhD immunoglobulin (RhD Ig), reported to Blood Matters Serious Transfusion Incident Reporting (STIR) voluntary haemovigilance program, and recommendations developed.

Method

STIR collects de-identified data using standard definitions and case report forms. Reports are validated by transfusion experts. Data is aggregated and recommendations made to assist clinical staff education. From January 2015 STIR included reports of RhD Ig procedural incidents.

Results

Over 2 years, 21 RhD Ig investigations were returned from 8 health services.

	Inappropriate administration	Omitted dose	Error in ordering/request	Storage/handling	Wrong dose	Total
Antenatal prophylaxis	2	5		1	1	9
Post sensitising event	4			1		5
Post-natal	5*	2	1			10
Total	11	7	1	2	1	22

Note *One report relates to a patient receiving unnecessary ante and post-natal doses.

Inappropriate administration includes RhD Ig administered to: RhD positive woman (4); RhD negative woman with RhD negative baby (2); RhD alloimmunised woman (1); different patient than product dispensed for (4). Of these categories, in the first 3 the women received a product they did not need, with the associated risks. Although the allo-immunised woman was not put at risk by the unnecessary administration of RhD Ig, her baby developed severe anaemia because her immune anti-D was not detected. In the last category, all patients receiving the product did need RhD Ig.

Most omitted doses were for antenatal prophylaxis, often involving patients with complicated pregnancies or social histories. There has been no indication of antibody development in the reports.

Conclusion

Reporting to STIR haemovigilance program highlighted RhD Ig errors to a wider audience. Opportunities exist in both antenatal and postnatal settings for practice improvement and risk reduction. Further education of clinical and laboratory staff would be beneficial. STIR will continue to monitor and report these incidents to help health services improve patient care.

327. An attempt to tailor wastage of blood products in the form of returned units

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Aim

Wastage of blood products may have an atrocious outcome on health care system. With an improvement in overall health care system there is an emerging focus on limiting the cost and avoiding the practices which may lead to wastage of blood products. It is time to call for adopting the practices which minimize the wastage of such prized blood units to almost zero.

Method

Retrospective audit was conducted in the section of blood bank from Jan- Dec2016 designed to determine rate of products returned to blood bank after dispatch, as a part of institutional wastage-reduction program. Reasons of return and key personnel and areas involved were identified.

Result

3999(46.5%) of blood components were wasted in study duration out of which returned blood component after specified time contributed 11.6%(n=467). 3.2%(n=15) blood products were returned within 30minutes and were taken back in inventory. Total number of returned products after 30minutes comprised of 67.4%(n=315) PRBC, 16.2% (n=76) Platelets, 11.1%(n=52) and Fresh frozen plasma (FFP). Majority of these products were returned from Operating room 34%(n=159). In 93%(n=109) cases, nurses and physicians were oblivious of the acceptable duration of keeping blood products out of controlled temperature. For total of 116 patients, ordering multiple units at a time with an anticipation of excessive bleeding during procedure (37.9%,n=44), patient's apprehensions (24.1%,n=28) and unavailability of proper intravenous access (19.8%,n=23), were identified as key reasons behind delaying the transfusion.

Conclusion

A multi-faceted plan was formulated as an important step towards hemovigilance in order to reduce the wastage in the form of small group educational sessions, flyers, new blood bag tags and transport box labels with 30minutes rule messages on them. A re-audit is now planned to analyze the affect and outcome of these efforts.

328. Post donation notification, counseling and response rate of reactive blood donors

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Aim

Provision of safe and adequate blood is a fundamental part of blood bank services. A crucial step in the prevention of transfusion transmitted infection is to notify and counsel reactive donors blood donors. Post donation notification and counseling of sero-positive blood donors not only protect the health of the donor but also prevent secondary transmission of infectious diseases. The aim of the study was to determine the response rate of reactive blood donor after notification of their screening status

Methods

This is an observational study carried out in Patel Hospital Blood bank over a period of 05 months from July – November 2016 involving total 1539 donors. All sero-positive blood donors were informed by the blood bank staff about an abnormal test result with an advice to report to blood bank for counseling and for referral to respective department/clinics of the hospital for further management. The response rate of reactive donors after notification of their abnormal test results was evaluated.

Results

The total reactive donors were 82(5.3%). 54(66%) reactive donors could be contacted of which 39(72%) responded positively to the notification calls and attended counseling at the blood bank and 28(34%) reactive donors could not be contacted either due to incorrect/changed contact details or did not picked up call even after three attempts.

Conclusion

The response rate of the reactive donors was found to be 72%. The response rate of reactive blood donors in developing countries is quite low suggesting insufficient health care knowledge and a poor understanding of screening tests.

330. A revealing discrepancy between ordering and use of fresh blood products in Cardiac Surgery

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Aim: To audit the fresh blood product requests associated with coronary artery bypass graft (CABG) surgery and primary aortic valve replacement (AVR) surgery, and to correlate this with eventual transfusion of these components, with the goal of identifying potential product wastage and financial loss.

Method: A single centre, retrospective study of CABG and AVR surgery performed over the 6 month period December 2016 to May 2017. The ordering of fresh blood products was collected from the transfusion medicine log book. Transfusion records were extracted and filtered by patient to blood use in the operating theatre and ≤ 6 hours post-operative. The difference between ordered blood products and transfused blood products were classified as “Not used” rather than wasted, as some re-entered circulation.

Result

	Primary CAGS: 50 cases					Primary AVR: 35 cases				
	Ordered	Used	Not used	Not used %	CT Ratio	Ordered	Used	Not used	Not used (%)	CT Ratio
RBC	52	16	36	69%	3.25	RBC	30	6	20%	5
Platelets	37	18	19	51%		Platelets	41	17	41%	24
FFP	8	5	3	37%		FFP	47	38	81%	19%
Cryo	13	4	9	69%		Cryo	57	51	89%	11%

The results demonstrate high ordering patterns when compared to usage particularly for red cells, platelets and cryoprecipitate (CAGS only). The high Crossmatch-Transfusion ratio of 3.5 and 5 for CABG and AVR surgery respectively is much higher than the benchmark of 1.5-2.5 (Steven M. Frank, 2013).

CAGS AVR

Product	Number of patients transfused	Product	Number of patients transfused
Red cells	4/50 (8%)	Red cells	4/35 (13%)
Platelets	16/50	Platelets	7/35
FFP	3/50	FFP	7/35
Cryo	2/50	Cryo	7/35

Patients not transfused 30/50 (60%) Patients not transfused 23/35 (65%)

Despite these procedures being classified as high bleeding-risk, 60-65% of patients did not require any transfusion - highlighting the need to better identify these patients as low to intermediate need to requiring transfusion.

Conclusion: This audit demonstrates that fresh blood products are being over ordered when compared to the usage rates. This reflects potential wastage of a scarce resource, increased workload and activity based cost of transfusion, including cross-matching, thawing and dispensation of fresh blood products. These results will form the basis of multi-disciplinary project to develop an algorithm for rational blood ordering which includes standardized Multiplate and ROTEM testing for all major cardiac surgery.

331. Anti-Jk3, an easy antibody to identify but an increasingly hard one to support.

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Over the past twelve months the Australian Red Cross Blood Service (ARCBS) has supported multiple patients with anti-Jk3. From January to June 2017, there have been three new cases in NSW, two of which were identified following suspected delayed transfusion reactions.

The majority of the patients presenting with anti-Jk3 are pregnant and two fresh units of Jk(a-b-) blood are usually requested to support delivery. Other patients with a historic anti-Jk3 will often present at the same time with urgent blood needs.

While this antibody is usually easy to identify in the laboratory, supporting these patients has become increasingly difficult as the number of new patients with anti-Jk3 continues to increase but available Jk(a-b-) donors remains stagnant. Often patients can only be supported with blood collected in another state, requiring transport of this rare blood across the country.

A transfusion plan for all patients with anti-Jk3 is essential to ensure relevant medical staff are aware of the potential problems with blood supply. This was highlighted earlier this year when two fresh Jk(a-b-) units collected for a pregnant patient expired in storage while the pregnancy progressed beyond 41 weeks gestation. This resulted in a subsequent request to supply a third unit of rare Jk(a-b-) blood urgently for delivery. This required thawing of a cryopreserved rare unit out of hours which was also subsequently not transfused. This outcome resulted in the waste of scarce and valuable resources.

An increase in donor phenotyping by the Blood Service over the last two years has only identified one additional Jk(a-b-) donor from the thousands of donors typed.

A more targeted approach to phenotyping will be taken in future as a report to identify country of birth has been developed, so ethnic groups can be targeted for particular phenotypes, eg. Jk(a-b-) is more common in Polynesians.

332. Prevalence and characteristic of irregular antibodies on myelodysplastic syndromes patients at NIHBT, Vietnam (2011-2016)

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Aim

“Study on prevalence and characteristic of irregular antibodies of myelodysplastic syndromes patients at National Institute of Hematology and Blood Transfusion (NIHBT) from 2011 to 2016”.

Method

984 myelodysplastic syndromes patients who were treated at NIHBT from 1/1/2011 to 31/12/2016 that were screening and identification irregular antibodies. Cross-sectional descriptive study. Using column agglutination technique and screening cell, panel cell which were made in NIHBT to detect irregular antibodies of patients.

Result

The prevalence of irregular antibodies of myelodysplastic syndromes patients was 6,2%, female: 6,9% and male: 5,5%; patients with multi-transfusion times had higher rate of irregular antibodies: 1- 4 times: 3,5%, 5-10 times: 7,4%, more than 10 times: 7,8%; patients had single and combined irregular antibodies was 72,1% and 27,9% respectively. Among patients with single irregular antibody, anti-E and anti-Mi^a were the most common (45,5%). Anti-E combined anti-Mi^a had the highest prevalence in patients had combined irregular antibodies group (53,8%). According by blood group system: Rh blood group: Anti-E was the most common (79,5%), anti-Mi^a had the highest prevalence (88,9%) in MNS blood group.

Conclusion

The prevalence of irregular antibodies of myelodysplastic syndromes patients was 6,2%; The prevalence of irregular antibodies of female was higher than male: 6,9% and 5,5%; patients with multi-transfusion times had higher rate of irregular antibodies (1- 4 times: 3,5%, 5-10: times: 7,4%, more than 10 times: 7,8%); patients had single irregular antibodies accounted for higher proportion than patients had combined irregular antibodies (72,1% and 27,9%). Anti- Mi^a (MNS system) and anti-E (Rh system) were had the highest prevalence in single antibody group (45,5%). Anti-E combined anti-Mi^a had the highest prevalence in combined antibodies group (53,8%). Anti-E was the most common of Rh blood group (79,5%), anti-Mi^a was the most common of MNS blood group (88,9%).

333. Transition to Version 3 of Criteria for the clinical use of intravenous immunoglobulin in Australia

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The *Criteria for the clinical use of intravenous immunoglobulin in Australia* (the *Criteria*) – *second edition* (2.1 BloodSTAR) is currently subject to an extensive review, public consultation and approval process for an update to Version 3 (V3).

A major transition strategy including a comprehensive communication plan is required to ensure appropriate transitioning of existing patients to the new version, as well as accommodating new patients under Version 3 within BloodSTAR. The objectives of the transition project are:

- Plan, communicate and facilitate patient transition to the *Criteria* V3;
 - a. Implement the *Criteria* V3 into BloodSTAR successfully;
 - b. Transition patient records within BloodSTAR 3.0 nationally.

There are 61 conditions and 89 indications in V2.1 *Criteria*. These have been mapped to the 54 conditions and 109 indications in V3 *Criteria*. The V3 *Criteria* indications that allow ongoing treatment are flagged as those requiring more detailed analysis, and potentially involve the transition of over 10,000 patient records. In addition, to accommodate V3 *Criteria*, a major upgrade to functionality of BloodSTAR is in development and is required for step-up to V3 *Criteria*.

Identification of the implications for stakeholders will require analysis of changes to the *Criteria* and subsequent modifications to BloodSTAR, including understanding the perspectives of a range of stakeholders including:

- patients (e.g. may need to see a different specialist),
- prescribers (e.g. may need to provide greater detail),
- nurses (e.g. may need to explain changes to patients)
- authorisers (e.g. will need to be aware of new requirements),
- those performing data analysis (e.g. mapping of patients between versions and changes to indications), and
- supply planners (e.g. possible changes to supply and demand forecasting).

All stakeholder groups will be consulted as part of the communication planning process.

334. Revision of the Criteria for the Clinical Use of Intravenous Immunoglobulin (IVIg) in Australia

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IVIg is a precious biological product and its use should be consistent with the evidence base and prescribed for the treatment of patients who are likely to benefit from immunoglobulin therapy, and for whom there are no safe and effective alternative treatments. The *Criteria for the Clinical Use of Intravenous Immunoglobulin (IVIg) in Australia (the Criteria)* was first published 2007 to assist clinicians and transfusion medicine professionals to identify the conditions and circumstances for which the use of intravenous immunoglobulin (IVIg) is appropriate and funded under the National Blood Agreement.

The Criteria was partially reviewed in 2012 and an additional complete review of *the Criteria* commenced in 2014. The development and review of *the Criteria* has involved the National Blood Authority (NBA) working with expert committees of specialist clinicians, to ensure that recommendations about access to publicly funded Ig products are based on best available evidence and clinical consensus opinion.

The revision process involves phases for:

- review and revision by expert specialist working groups
- recommendation of changes to the National Immunoglobulin Governance Advisory Committee (NIGAC)
- public consultation, and
- approval by the Jurisdictional Blood Committee (JBC) representing all Australian governments

The first round of proposed changes that were approved by the JBC in 2015 related to 32 conditions that represent around 96% of current Ig use. The second round of proposed changes relates to 31 conditions that represent around 4% of publicly funded Ig use. These will be considered for endorsement by JBC in September 2017.

Once endorsed, the revised *Criteria* will be implemented through BloodSTAR, at the conclusion of the pre-implementation and transition phases.

The pre-implementation phase is underway with transition strategies being developed to ensure appropriate implementation and communication of the proposed changes to stakeholders to assist with implementation of the revised *Criteria* (currently planned for 2018).

335. Evidence-based national clinical practice guidelines for blood, blood products and blood related services in Australia

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Aim

To describe the evidence-based clinical practice guidelines for blood, blood products and blood related services that have been finalised as well as those under development to underpin best practice in the management and use of those products and services in Australia.

Method

The National Blood Authority (NBA) has completed the suite *Patient Blood Management (PBM) Guidelines*, comprising six modules: Critical Bleeding/Massive Transfusion (2011), Perioperative (2012), Medical (2012), Critical Care (2013), Obstetrics and Maternity (2015) and Neonatal and Paediatrics (April, 2016) as well as the *Guidelines for the management of haemophilia in Australia* (July, 2016).

The NBA also recently commenced the review and update of the *Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* (2003) and the PBM Guidelines. The update processes will incorporate a review and assessment of methodologies capable of retaining the currency of guidelines into the future. Upon identification and implementation of the preferred methodology, it is expected that updates to the NBA's clinical practice guidelines will be triggered as the evidence base and clinical practice evolves.

Results

The clinical practice guidelines managed and published by the NBA to date have been commended within Australia and internationally. In particular, the PBM Guidelines and have contributed to significant improvements in clinical practice and a reduction in the number of red blood cell transfusions, exemplified in the demand for red blood cells falling by more than 21% since 2011-12.

Conclusion

By continuing to improve and streamline the development and update process of clinical practice guidelines, the NBA is seeking to ensure that they will continue to underpin best clinical practice in relation to the appropriate use of blood and blood products. Preliminary findings on the review and assessment of methodologies for a more sustainable and updateable guideline model are expected in December 2017.

336. Sickle cell disease: Advantages of phenotype compatible red cells and utility of genotyping in erythrocytapheresis

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Background

Red cell transfusion and red cell exchange (RCE) have improved outcomes for sickle cell disease (SCD) patients, but carry the risk of alloimmunisation. There are no universally accepted transfusion guidelines for these patients. The efficacy of providing phenotypically compatible red cell units in reducing alloimmunisation, and role of genotyping remains poorly understood.

Aims

To document the alloimmunisation rate for SCD patients undergoing RCE with red cells phenotypically compatible for clinically significant antigens, and to consider the utility of genotyping.

Methods

A retrospective audit was performed on all SCD patients undergoing RCE at a quaternary referral centre between January 2010 and June 2016. Data on blood group, serologic phenotype, genotype, and historic antibodies were collected. For each RCE episode, antibody screening results and number of units transfused were recorded. Patients received red cells phenotypically compatible for ABO, Rh, Kell, Kidd and Duffy antigens. Units compatible for MNSs antigens were provided to patients who phenotyped as Fy(a-b-).

Results

Twenty-nine patients underwent RCE. Sixteen patients (55.2%) had a history of alloimmunisation at their first RCE episode. Seven patients (24.1%) developed at least one new antibody, one was clinically significant (anti-C^w). C^w was not on the genotyping panel. Fourteen patients (48.3%) had at least one known antibody that was not detected in at least one antibody screen. Genotyping information was available for twenty-six patients, six had a GATA box mutation.

Conclusion

Our results demonstrate a high alloimmunisation rate in SCD patients. Despite extended phenotype matching of red cells, 25% of patients formed additional alloantibodies. Notably, only one was clinically significant. The available genotyping information could not have prevented this. Genotyping was valuable in extending the donor pool available to patients who serologically phenotyped as Fy(a-b-) but had a GATA box mutation. The data also highlight the dangers of evanescent antibodies and support calls for national/regional alloantibody databases.

337. RhD variant DAR on the red cells of an antenatal patient

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Aim

To determine the RhD status of a 25 year old antenatal patient whose red cells showed discrepant reactions with routine monoclonal anti-D reagents.

Method

Routine blood group and antibody screen was requested on a 25 year old antenatal patient (G1P0) at 37 weeks gestation. Initial testing performed on the Ortho AutoVue® Innova identified weak RhD agglutination. Subsequent testing was performed by BioRad CAT (Table 1). The AlbaClone® Advanced Partial RhD typing kit (Alba Bioscience, Scotland) was utilised to differentiate weak D from partial D. Molecular testing was performed by the ARCBS Red Cell Reference Laboratory using BioArray RhD BeadChip.

Results

Initial testing by BioVue CAT on the Ortho AutoVue® Innova system showed weak agglutination (grading 1) with the anti-D clone D7B8. RhD agglutination was not observed with BioRad CAT using different clones of anti-D. Table 1 summarises the reactivity pattern of monoclonal anti-D reagents used in the initial determination of RhD status. Results from the AlbaClone® Partial RhD kit suggested RhD was a partial D, DFR. However, subsequent RhD genotyping from ARCBS identified the RhD as partial D, DAR.

Anti-D Reagent	Anti-D Clone	Method	Grading
BioVue ABO-Rh/ Reverse grouping	D7B8	BioVue CAT - Ortho AutoVue® Innova	1
DiaClon ABO/D (VI-) + reverse grouping	LHM 59/20 (LDM3) / 175-2	BioRad CAT	0
DiaClon ABO/Rh (VI+)	ESD-1M + 175-2		0

Table 1. Reactivity patterns of monoclonal anti-D reagents (Grading 0-4).

Conclusion

This case highlights the value of utilising different clones of monoclonal anti-D to elucidate RhD status and to differentiate between weak D and partial D. However, molecular testing may be necessary to determine the RhD phenotype. DAR was described in 1998 and is caused by single nucleotide polymorphisms on exons 4, 5 and 7 of the RHD gene. DAR belongs to the weak D type 4 cluster of alleles characterised by an F223V amino acid substitution at the extracellular portion of the RhD protein. Patients with the partial RhD DAR have the potential to make immune anti-D. They should therefore be treated as though RhD negative, and during pregnancy, be administered routine RhD-Ig prophylaxis.

NOTE: No Conflict of interest to disclose.

338. Blood transfusion and paediatric orthopaedic surgery

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Cerebral palsy (CP) is a collection of permanent, non-progressive disorders affecting movement, muscle tone and posture. Corrective orthopaedic surgery is an integral component in their care, to maintain optimal mobility and function. Corrective spine, hip and lower limb extremity surgery places these patients at risk of major blood loss and red blood cell (RBC) transfusion. There is no literature specific to this cohort and their need for RBC transfusion during surgery.

Aim

To review factors contributing to allogeneic RBC transfusion, in children with CP undergoing major orthopaedic surgery.

Methods

A retrospective review at a tertiary paediatric hospital of all patients with CP undergoing primary spinal fusion or single event multi-level surgery (SEMLS) between January 2010 and December 2015. Data collected included: demographics, co-morbidities, medications, surgical details, laboratory investigations, cell salvage and transfusion rates.

Results

134 children were included in analysis; 98 patients undergoing SEMLS surgery (pelvic and/or femoral osteotomies), 36 patients undergoing primary posterior spinal fusion. Twelve percent (16/134) of patients were anaemic pre-operatively and only 19% (25/134) of patients had ferritin assessed preoperatively.

Forty-nine patients (37%) received a RBC transfusion either intra- or post-operatively. Intra-operative usage of tranexamic acid and cell saver was 89% and 81% respectively for the scoliosis group and 22% and 3% for the SEMLS group. Univariable analysis showed GMFCS level, PEG feeding, sodium valproate usage, surgical time, spinal fusion, pelvis involvement, and pre-operative Hb level <115g/L were significantly associated with transfusion. Multi-variable analysis demonstrated that GMFCS level and longer surgical times were independently associated with transfusion risk.

Conclusion

More than one third of CP patients undergoing major orthopaedic surgery received a RBC transfusion. Patients who were more severely affected with longest surgery duration were at highest risk. Significant improvements can be made in pre-operative assessment and management of anaemia and iron deficiency.

339. Increased survival of antigen negative red cells in a case of warm autoimmune haemolytic anaemia

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Background

In warm autoimmune haemolytic anaemia (WAIHA), haemolysis is mediated by autoantibodies binding to “self” antigens on the red blood cells (RBCs) at 37°C. Warm autoantibodies typically react against all RBCs but can demonstrate apparent specificity showing enhanced reactivity when a particular blood group antigen is present, most commonly a Rh antigen.

Case Study

A 31 year old female presented with symptomatic anaemia. Laboratory testing showed a haemoglobin (Hb) level of 46g/L with peripheral blood and biochemical markers consistent with haemolysis. Pre transfusion testing showed a strongly positive direct antiglobulin test against IgG(4+) and the presence of an auto anti-e like antibody that reacted strongly (4+) with e positive cells but did not react with e negative cells. Her predicted phenotype was CCDee (R1R1), which was subsequently confirmed by genotyping. Absorption procedures revealed no alloantibodies.

19 units of R1R1 phenotype matched red cells were transfused without improvement in Hb. Treatment modalities that were trialled included high dose steroids, IVIg and rituximab however laboratory evidence of red cell haemolysis persisted until day 16. A trial of ccDEE (R2R2) units was commenced as it was predicted that these red cells may survive longer in vivo, outweighing the risk of alloimmunisation. Post commencement of R2R2 cells the Hb levels steadily increased and transfusion requirements declined indicating that the e negative units had increased survival in vivo when compared to the e positive units (figure 1) despite persistent red cell haemolysis.

Figure 1

Daily haemoglobin level post transfusion of R1R1 and R2R2 red cells.

Discussion

There is limited data confirming that the transfusion of antigen negative red cells can increase red cell survival in the recipient where autoantibodies appear to have an absolute specificity for a blood group antigen. The ANZSBT guidelines recommend transfusing phenotype matched red cells in WAIHA cases to reduce the risk of alloantibody formation however there are no recommendations for autoantibodies with apparent specificities¹. This case demonstrates an autoantibody with an apparent specificity where the transfusion of antigen negative red cells proved more effective in the treatment of anaemia than phenotype matched red cells, resulting in a clinically significant decrease in transfusion requirements.

References

1. Australian and New Zealand Society of Blood Transfusion (ANZSBT). Guidelines for transfusion and immunohaematology practice. ANZSBT 1st Edition, 2016

340. Differing response to IVIg products in a patient with chronic anaemia secondary to parvovirus infection

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Background: Parvovirus B19 (PVB19) is a single stranded DNA virus which normally causes a brief erythropoietic aplasia however due to the long RBC lifespan, significant anaemia is not seen. In patients who are immunocompromised, chronic infection can progress to anaemia. Replacement of antibodies through Intravenous Immunoglobulin (IVIg) is considered a first line treatment for severe anaemia due to chronic PVB19 infection in immunocompromised patients¹.

Case Study: We report a 48 year-old male who progressed to chronic anaemia following confirmed PCR positive PVB19 infection in 1999. Initially commenced on Intragam[®]P, he has been given multiple IVIg products due to product shortages, recalls and treatment decisions. It was noted his red cell transfusion requirements were inconsistent prompting a review of red cell requirements and possible correlation to the IVIg product (table 1).

Table 1: Average patient red cell transfusions per month when receiving different IVIg products

Product	Date (Month/Year)	Average units transfused per month
Octagam [®] 5%	Jan 06 - Sep 10	0.1
Intragam [®] P	Oct 10 - May 12	3.1
Octagam [®] 5%	June 12 - Sep 13	0.3
Octagam [®] 10%	Oct 13 - May 14	1.3
No product	Jun 14 – Aug 14	3.3
Octagam [®] 10%	Sep 14 -Feb 16	2.0
Privigen [®] 10%	Mar 16 - Oct 16	3.5
Flebogamma [®] 10% DIF	Nov 16- April 17	4.0

Discussion: While red cell requirements are increasing overall, each time he was restarted on an Octagam[®] product the red cell usage declined for the period of Octagam[®], in particular Octagam[®]5%, suggesting Octagam[®] is the most effective IVIg product for this patient. Due to changes in imported IVIg arrangements, Octagam[®] is no longer available in Australia and the patient was transitioned to Privigen[®] in March 2016. A six month trial of Privigen[®] followed by six months of Flebogamma[®]10% did not reduce red cell requirements. The patient has recently started Intragam[®]10%. PVB19 antibody titres for IVIg products are rarely published nor included in IVIg product comparison publications except for Octagam[®]5%, published as typically containing >180IU/mL, stated as several times higher than the antibody titre required in IVIg products.

Conclusion: We conclude that there is a large variation in the total PVB19 antibody titre between IVIg products, presumably related to the donor cohort. Products with higher PVB19 antibody titres could provide a more effective treatment for chronic PVB19 infection. With increasing worldwide demand for IVIg treatment our hope is that in the future, disease specific immunoglobulin product data and recommendations will be available to obtain the best patient outcome.

References

- National Blood Authority (NBA) *Criteria for the clinical use of Intravenous Immunoglobulin in Australia*. NBA 2nd edition, 2012.

341. 10 years of elearning in improving transfusion practice and implementing PBM in Australia (2007-2017)

English L¹, Clark T¹, Ogley S¹, Verrall T¹, Thomson A¹, Peterson D¹

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Background

BloodSafe eLearning Australia (BEA) (www.bloodsafelearning.org.au) is celebrating 10 years of delivering eLearning in 2017.

BEA is an online educational program designed to increase knowledge of patient blood management and clinical transfusion practice with learning aligned to Australian guidelines and standards, published evidence and expert consensus. BEA delivers 17 courses that utilise a range of learning materials including videos of experts discussing and/or demonstrating best practice, interactive learning and case scenarios.

Aim

To undertake an evaluation of BEA courses to determine if the program is meeting its objectives of providing knowledge that can be put into practice to improve patient outcomes.

Method

A retrospective analysis of 10 years of data including formal evaluations, course completion statistics and questionnaires was undertaken to investigate the perceived quality, value and effectiveness of the BEA courses and how this affects clinical practice.

Result

Analysis of learner registration and course completion data shows that as of 30 June 2017 BEA has:

- >417,160 registered learners
- >846,586 courses completed

Analysis of 3,885 learner evaluation questionnaires showed that users believe the program:

- Increases knowledge – 89% of respondents
- Will change their clinical practice – 62%
- Assist them to identify near misses and adverse events – 84%
- Improve patient outcomes and safety – 88%

Actions and changes identified by users to improve outcomes and safety include: reviewing local policies to align with guidelines, more appropriate use of blood, development of massive transfusion protocols, better assessment and management of postpartum haemorrhage, and providing staff with further education in transfusion practice.

Conclusion

BEA has played an important role in improving clinical transfusion practice and implementing patient blood management in Australia. The program provides users with credible, consistent knowledge that they can apply to their clinical practice.

344. ISBT 128 Component Codes For Australian Blood Products

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The Australian Red Cross Blood Service will be adopting the ISBT 128 standard for the labelling of blood components as described in the National Blood Authority's barcode specifications paper 'Barcode Specifications for Blood and Blood Products Funded under the National Blood Arrangements'. By moving to this standard, all manufactured blood components will be registered in the ISBT 128 Product Description Code Database which is an international register managed by ICCBBA².

The ISBT128 defines the naming convention of each component code by a product hierarchy (Category, Subcategory, Class, Modifiers and/or Attribute). The **Category** relates to the general heading such as blood, tissues, ocular therapies etc. The **Subcategory** allows for classification within the Category. An example of a Subcategory for blood includes Red Blood Cells, Platelets, and Plasma. The **Class** level is the description used in labelling which is then further described using **Modifiers and/or Attributes**.

The description of each component in the Product Description Code Database is standardised; however the text that is displayed on the actual component label is decided upon by the specific organisation. This allows for differences in languages and regulatory requirements within each country. This 'database' provides registered facilities the ability to source product information about components they may receive from overseas without the challenge of understanding the eye readable text in another language.

Issuable blood components will use internationally recognised codes. The current codabar component code for Red Cells in SAG M LD is 04390. The ISBT 128 equivalent code will be E8770V00. Where, 'E' defines it as a blood product, 'V' signifies voluntary donor. ISBT128 also prescribes the format of component codes when defining divisions or splits which is defined by the '00'. The first or second level of division – depending on the times divided, is encoded within the component barcode data structure allowing for health providers to record electronically which division has been used from the parent component rather than by eye readable text only.

Australian Red Cross Blood Service is funded by Australian Government's

¹ International Council for Commonality in Blood Banking Automation.

346. Improving the safety of blood products retrieved from unmanned blood fridges in the after-hours setting.

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Aim

The aim was to implement guidelines and a training program to facilitate the ability of clinical staff to safely and appropriately access emergency blood products and pre-crossmatched red blood cells in the after-hours setting in hospitals that did not have 24-hour Pathology services.

Method

Those staff who would be responsible for accessing the after-hours blood fridge would be identified by the Director of Nursing & Midwifery at the facility, and would be given access to specific training to ensure that they would be able to access the right product at the right time, and guarantee that the required paperwork be completed to ensure traceability of the product. This training was based on guidelines written in conjunction with Pathology to ensure adherence to accreditation requirements.

The training program was developed by the Clinical Nurse Consultant for Transfusion Medicine alongside the Network Scientist for the Pathology service to ensure educational, scientific and clinical relevance.

Relevant staff members from those facilities within the local health district which did not have a 24 hour Pathology service were targeted and given access to training with the Clinical Nurse Consultant and Supervising Scientist.

Result

A pre- and post- training session knowledge test was undertaken by the participants, demonstrating a marked increase in knowledge acquisition regarding safe blood supply and use after attendance at the training session.

Clinical incidents relating to the after-hours retrieval of blood products by non-pathology staff have decreased, although this will continue to be monitored to determine the longitudinal effect of this training program implementation.

Conclusion

By working in conjunction with Pathology partners and providing an educationally sound training program for relevant clinical staff, we can help ensure the safety of blood supply after-hours to critically unwell patients without compromising quality and safety.

"No conflict of interest to disclose".

347. Hospital data reports for benchmarking and practice improvement: Australian and New Zealand Massive Transfusion Registry

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Background/Aim

The Australian and New Zealand Massive Transfusion Registry (ANZ-MTR) collects comprehensive data on adults receiving massive transfusion (MT), defined as ≥ 5 red blood cells (RBC) within any 4-hour period. Complete data are now available for $>5,000$ cases from 25 hospitals. Patient populations and clinical practice vary between regions. To support benchmarking and practice improvement activities, we developed site-specific MT hospital data reports (HDRs) as a quality tool.

Method

ANZ-MTR data were analysed and charts/tables produced for each site compared to peer hospitals, Australia, NZ and overall, using statistical analysis software. An electronic HDR template with a script to automatically populate the individual reports with site-specific data was developed. Pilot sites were selected to test the new HDR and feedback obtained.

Results

The HDR presents data in formats suitable for a wide range of personnel, including clinical staff, laboratory scientists and senior management. Data include: 1) patient characteristics (age, sex, hospital length-of-stay, ICU admission); 2) clinical bleeding contexts; 3) in-hospital mortality (unadjusted and adjusted); 4) transfused blood components, including ratios; 5) blood product use in larger volume MT cases (i.e. ≥ 10 RBCs within 24-hours of MT onset); 6) laboratory data. Individual HDRs include a customised executive summary. In response to feedback, a visual pictogram summary of overall MTR data accompanies each HDR.

Conclusion

Detailed individual hospital information about MT patient care and outcomes, along with peer-hospital comparative data, may stimulate practice review including assessment against national standards and patient blood management principles.

348. Donor haematology lessons for choosing testing wisely

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In healthcare, the “choosing wisely” program promotes discussions on which medical tests and procedures are needed. Lean manufacturing emphasises the elimination of “Muda” (futility, uselessness and wastefulness). Donor testing within the Australian Red Cross Blood Service offers opportunities from the implementation of these philosophies.

Aim

To determine the impact of the cessation of routine annual donor haematology testing and full blood count (FBC) point of care testing at plateletpheresis collection sites.

Method

First, to determine if annual FBC testing could be removed without losing the benefit of detecting abnormal FBC findings, 313,360 FBC tests from 47,592 blood donors were reviewed. Second, analysis of whether haematology analysers could be decommissioned from donor centres and replaced with the donors historical platelet counts for programming apheresis platelet collections was conducted. Removal of the analysers would eliminate the requirement for machine maintenance which is primarily conducted by nursing staff.

Results

Regulatory review confirmed annual FBC testing could be removed as a mandated requirement. The review of the FBC tests provided evidence of the infrequency of abnormal FBC findings and provided scant useful data when abnormal counts were detected. Furthermore, the serial testing data on the 47,592 donors confirmed that there was minimal clinical impact of apheresis donation on an individual’s lymphocyte and platelet counts.

Available data from the Blood Service confirmed the robust performance of historical mean platelet counts for programming platelet donation. Donor platelet counts are reliably predicted from mean historical results. Following the removal of haematology analysers, national quality control data confirmed the maintenance of platelet yields.

Conclusion

Significant productivity improvements in donor haematology testing were attained whilst maintaining donor safety within existing regulatory requirements.

349. A case of neonatal jaundice due to an antibody to low incidence antigen(s)

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Background

The presence of antibodies to low incidence red cell antigens can cause Haemolytic Disease of the Fetus and Newborn. Such antibodies are not detected by routine antibody screening tests, so can cause diagnostic difficulties.

Case Report

A 38-year-old South African woman, with no previous history of blood transfusions or surgeries delivered at 37 weeks gestation. Routine antenatal testing had been performed and the mother was O Rh(D) Positive with no antibodies detected. The baby was small for gestational age and jaundiced. Testing on the baby showed her to be A Rh(D) Positive with a Positive DAT. ABO incompatibility was investigated, but ABO antibodies could not be eluted from the neonatal cells. The neonatal eluate reacted strongly with paternal red cells.

Maternal, paternal and neonatal blood were sent to the Red Cell Reference Laboratory at the Blood Service - Victoria. The maternal plasma were reactive with paternal red cells. The plasma was negative when tested against a more extensive panel of red cells expressing low incidence antigens. Further testing showed probable anti-STEM and/or anti-DAK. Phenotyping of the baby's cells could not be performed due to lack suitable antisera, DNA sequencing indicated that the mother was negative for both the STEM and DAK antigens and the neonate and father were both RH:49(STEM+) and RH:54(DAK+). Approximately 4% of Africans are STEM and DAK positive.

Conclusion

When neonatal haemolysis cannot be explained by ABO or commonly detected maternal antibodies, clinicians must be alerted to suspect the presence of antibodies to low incidence antigens. This investigation is important for monitoring of further pregnancies because specific testing with antigen positive cells or ultrasound fetal monitoring will be required, as routine antenatal testing will not demonstrate any antibodies.

350. Cryopreservation of platelets activates pathways associated with apoptosis

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Aim: Cryopreservation of platelets induces a significant increase in exposed phosphatidylserine and the release of phosphatidylserine-expressing microparticles. Phosphatidylserine is associated with both activation and apoptosis and may mediate the procoagulant function of platelets. The aim of this study was to examine the apoptotic profile of cryopreserved platelets.

Methods: Buffy coat-derived platelets were frozen at -80 °C with DMSO (5% final concentration). Cryopreserved platelets (n=8) were thawed at 37 °C, reconstituted with a unit of thawed plasma. Paired units were tested prior to freezing and after thawing (0, 6 and 24 hours post-thaw) for *in vitro* markers of apoptosis, using flow cytometry, a proteome profiler apoptosis antibody array (R&D Systems) and western blotting. Data were analysed using a one-way ANOVA, with p<0.05 being considered statistically significant (*).

Results: Phosphatidylserine externalisation was significantly increased in cryopreserved platelets, as measured by lactadherin and annexin-V binding. The abundance of apoptotic proteins of the intrinsic pathway, including Bax, cytochrome c and SMAC were increased in the cryopreserved platelets. The expression of several death receptors, including FADD were altered by cryopreservation. Mitochondrial damage was evident in the cryopreserved platelets, as measured by a reduction in tetramethylrhodamine (TMRE) sequestration. Interestingly, several proteins associated with modulating oxidative stress (catalase, heme oxygenase-1, heme oxygenase-2) were altered in cryopreserved platelets, resulting in increased intracellular ROS (carboxydichlorofluorescein [DCF] staining) and superoxide anion formation (dihydroethidium [DHE] staining). Finally, cleavage of the effector proteins, caspase-3 and caspase-9 was observed in the cryopreserved platelets after 24 hours of post-thaw storage.

	Fresh	Post-thaw 0 h	Post-thaw 24 h
Annexin-V (% positive)	1±0	67±4*	45±6*
TMRE (MFI)	1287±108	166±33*	482±107*
DHE (MFI)	105±8	218±16*	233±27*
Cleaved caspase-3 (MFI)	66±48	89±19	119±25*

Conclusion: Platelets acquire many features associated with apoptosis and increased procoagulant function following cryopreservation. Further work is required to determine the pathways leading to apoptosis and the enhanced procoagulant function of cryopreserved platelets.

Key words: Platelets, cryopreservation, phosphatidylserine, apoptosis.

Conflict of interest: The authors have no conflict of interest to disclose.

352. How often was iron deficiency managed in gastrointestinal bleeding?

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Aim:

Gastrointestinal bleeding (GIB) is common in clinical practices with heterogeneous etiologies. Apart from investigating for and managing the underlying pathologies, patients are often given blood transfusion. However, the associated iron deficiency anaemia is often overlooked. This study aims to look into how often iron deficiency is managed at the time of GIB patients received transfusion and after discharge.

Method:

Anonymous data of GIB patients was retrieved from a central database of public hospitals including age, number of units of red cell (RC) transfused, pre- and post-transfusion Hb; and the use of iron during hospitalization and upon discharge in 2016. 3 groups including GI neoplasms; non-malignant lower and upper GIB was analyzed. Co-morbid complications with cardiovascular or cerebrovascular were excluded.

Result:

	GI neoplasms	Non-malignant Lower GIB	Non-malignant Upper GIB
Number of unique patients	3,604	3,161	5,612
Median age (range)	72 (3~105)	78 (0~106)	75 (0~105)
Number of transfusion episodes	5,377	5,870	9,789
Units of RC transfused	9,179	10,262	16,558
Episodes			
• Pre-transfusion Hb absent	97 (1.8%)	14 (0.2%)	17 (0.2%)
• Post-transfusion Hb absent	909 (16.9%)	305 (5.2%)	497 (5.1%)
• Pre-transfusion Hb \geq 8 g/dL	1,423 (26.5%)	1,429 (24.3%)	2,043 (20.9%)
• Post transfusion Hb \geq 10 g/dL	1,626 (30.2%)	1,602 (27.3%)	2,379 (24.3%)
• Multiple units RC given	3,230 (60.1%)	3,521 (60.0%)	5,510 (56.3%)
Neither parenteral nor oral iron given	2,458 (68.2%)	2,352 (74.4%)	3,863 (68.8%)

Conclusion:

Despite the absence of detailed clinical information, a substantial proportion of transfusion episodes occurred at Hb \geq 8g/dL (4,895 (23.3%)) and 5,607 (26.7%) resulted in post transfusion Hb (\geq 10g/dL). More importantly, up to 8,673 (70.1%) transfused patients were neither given parenteral nor oral iron. The results indicate that transfusion appropriateness and iron deficiency management should be enhanced in clinical practice.

353. Improved outcomes and reduced costs associated with a health system-wide patient blood management program: a retrospective Western Australian observational study.

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Background

Patient blood management (PBM) programs are associated with improved patient outcomes, reduced transfusions and costs. In 2008, the Western Australia Department of Health initiated a comprehensive health-system-wide PBM program. This study assesses program outcomes.

Study Design and Methods

This was a retrospective study of 605,046 patients admitted to four major adult tertiary-care hospitals between July 2008 and June 2014. Outcome measures were red blood cell (RBC), fresh-frozen plasma (FFP), and platelet units transfused; single-unit RBC transfusions; pretransfusion haemoglobin levels; elective surgery patients anaemic at admission; product and activity-based costs of transfusion; in-hospital mortality; length of stay; 28-day all-cause emergency readmissions; and hospital acquired complications.

Results

Comparing final year with baseline, units of RBCs, FFP, and platelets transfused per admission decreased 41% ($p < 0.001$), representing a product cost saving of AU\$18,507,092 and between AU\$80 million and AU\$100 million in estimated activity-based savings. Mean pretransfusion haemoglobin levels decreased from 7.9 g/dL to 7.3 g/dL ($p < 0.001$), and anaemic elective surgery admissions decreased 20.8% to 14.4% ($p = 0.001$). Single-unit RBC transfusions increased from 33.3% to 63.7% ($p < 0.001$). There were risk-adjusted reductions in hospital mortality (odds ratio [OR], 0.72; 95% confidence interval [CI], 0.67-0.77; $p < 0.001$), length of stay (incidence rate ratio, 0.85; 95% CI, 0.84-0.87; $p < 0.001$), hospital acquired infections (OR, 0.79; 95% CI, 0.73-0.86; $p < 0.001$), and acute myocardial infarction-stroke (OR, 0.69; 95% CI, 0.58-0.82; $p < 0.001$). All-cause emergency readmissions increased (OR, 1.06; 95% CI, 1.02-1.10; $p = 0.001$).

Conclusion

Implementation of a unique, jurisdiction wide Western Australian PBM program was associated with improved patient outcomes, reduced blood product utilization, and product related cost savings.

354. Passenger lymphocyte syndrome with anti-B and anti-Jk^a post combined intestinal and renal transplant

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Aim/background

Passenger lymphocyte syndrome (PLS) is an immune mediated hemolysis in which antibodies directed against recipient red blood cell (RBC) antigens are formed by viable donor B-lymphocytes found within an allograft, leading to alloimmune destruction of recipient RBC.

Method

This is the first reported case of PLS after combined intestinal and renal transplant in the literature. A 47-year-old group B, D+, Jk(a+b+) male received an ABO minor-mismatch combined intestinal and renal transplant from a cadaveric donor who was group O D+. The recipient developed clinically significant hemolysis 18 days after combined intestinal and renal transplant with a hemoglobin nadir of 59g/L. He was supported with donor and recipient compatible blood components, with laboratory evidence of resolution of hemolysis by day 52 post transplant without requiring any additional interventions.

Result

Both the recipient and donor had a negative antibody screen immediately prior to transplantation. Two alloantibodies, anti-B and a weak anti-Jk^a, were detected in the recipient's eluate.

Conclusion

It is important to be vigilant about possible PLS post transplant especially in high-risk populations such as those undergoing intestinal and/or multivisceral transplant with minor ABO incompatible allografts. Although PLS is a rare and self-limiting condition, early recognition allows timely provision of compatible blood components as well as minimization of further harm from hemolysis.

355. ISBT128 implementation to critical ICT systems

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The Australian Red Cross Blood Service will be adopting ISBT 128 for labelling of all fresh blood components by June 2018 in accordance with the National Blood Authority 'Barcode Specifications for Blood and Blood Products Funded under the National Blood Arrangements'. The implementation of new data structures for donation numbers and component codes is complex and will mean significant change to information system applications, hardware and laboratory equipment whilst still ensuring Australia's blood supply to patients remains unaffected.

The Blood Service has identified 53 internal systems in use that will be impacted by this change. Of these systems, 32(60%) are managed and supported by the Blood Service staff in the Information, Communication and Technology (ICT) division. The remaining 18(34%) and 3(6%) are managed by the Manufacturing and Finance divisions respectively. The interactions of data flow and information between systems makes what appears to be a relatively simple change for ISBT128, complicated. The movement of mono-directional and bi-directional information is critical to operational processes during the collection, manufacture and issue of blood components. Any modifications to critical information systems are required to undergo stringent testing in line with the ICT Validation Framework to ensure the integrity and quality of data is maintained and that the Blood Service continues to meet its operational and regulatory responsibilities.

Due to the crucial importance of success, the ISBT128 system changes shall be managed in a **Build** [Design (DD), Development (DEV), Unit test (UT), Informal system test (ST)]; **Test** [Formal system test (ST), Formal system integration test (SIT), User acceptance test (UAT)] and; **Deploy** [Dry-run, Go-live, and Post go-live verification] **framework** to ensure a robust approach towards system deployment.

The 32 ICT system changes shall be deployed as 23 separate deployment events during July 2017 to June 2018. The final deployment event will be the transition of the National Blood Management System. All components collected after this time and manufactured shall be issued with an ISBT128 Transition label.

Australian Red Cross Blood Service is funded by Australian Government's

The Information Standard for Blood and Transplant (*ISBT128*) is the global standard for the identification, labelling and information transfer of human blood, cells, tissues and organs and other medical products of human origin.

356. Improving cold chain blood supply to theatre

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Aim

To improve cold chain traceability of red cells to theatres at a large tertiary hospital by trialling delivery in shippers with aim to decommission the theatre blood fridge and improve safety and traceability of blood stored outside of Transfusion laboratory

Method

Validated blood shippers and temperature data loggers were issued by the Transfusion laboratory to theatres as a 'point of care blood fridge' during operations where red cells were requested. The laboratory IT system provided traceability of the red cells, barcoded shipper and data logger. A comprehensive education program was developed by Transfusion Medicine for Porters and Theatre staff. The trial was audited against NSQHS Standard 7 criteria 7.7: Ensuring the storage of blood is consistent with best practice. The following data points were collected: Patient details, Theatre number, time issue and return of shipper, average hours in theatre, procedure type, number of units issued and transfused. Non-conformances were raised using the hospital incident management system.

Results: 8 week trial

Shippers	Hours in Theatre	Red cells issued	Red cells transfused
142 (mean 18/week)	Mean = 4	364 (median 2 / shipper)	133 (36%)

Non-conformance: n = 1 Shipper left in theatre after staff vacated

Blood waste: 0 units. All red cells returned within acceptable storage temperature (2-6°C)

Conclusion

We successfully trialled use of "point of care" blood in validated shippers in lieu of the Theatre blood fridge. The education program was effective in ensuring blood shippers were not relocated to wards with patients. Theatre staff reported the shipper were easier to manage than the requirements of documentation and maintenance of a blood fridge. The number of unused shippers arises opportunity to reduce the number of blood requests through education on blood utilisation. This quality improvement activity was endorsed for routine practice at end of trial.

357. Design and implementation of a patient blood management pilot program for orthopaedic surgery

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Background

We describe the design and implementation of a Patient Blood Management (PBM) pilot program for patients undergoing elective large joint replacement (EJR) surgery.

A 2012 internal audit revealed that pre-operative anaemia is present in 25% of patients undergoing EJR, they are more likely to receive transfusion (OR=4) and have a 3.4 day longer length of stay ($p<0.05$). Preoperative anaemia investigation, including assessment of iron status was poorly performed.

Aims

- Development of a collaborative multidisciplinary, multimodality PBM program for EJR surgery where estimated blood loss is $>30\text{g/L}$.
- 1. To create a systematic, visual display of the components of the process and develop clear communication and defined pathway for clinicians and patients.
- 2. Early identification and treatment of iron deficiency anaemia.
- 3. Improvement of pre-operative haemoglobi and consequent reduction in perioperative transfusion.

Method

A PBM working group, with the support of a committed hospital administration was formed and manned by a team which included an Orthopaedic surgeon, anaesthetist, haematologist, transfusion nurse, pharmacist and preadmission and perioperative clinic NUMs. Using clinical practice improvement methodology (CPI), facilitated by a Graduate Management trainee, a project plan including timeline was created.

Results

The patient process from initial surgical consultation to undergoing surgery was mapped out in a flow chart. Intervention points were defined where screening and treatment could take place and alternative patient flow pathway planned.

The timeline was created and included obtaining feedback from stakeholders, introducing patient/GP letters, developing data collection templates and conducting education sessions. A virtual clinic is run weekly by a haematology fellow and transfusion CNC. Obtaining engagement from appropriate clinicians was the most significant barrier to project launch.

Conclusion

The project highlights the importance to having a formal project design with timeline, process mapping and engaged key-players to successfully enact change. The assessment of clinical outcomes of anaemia, iron deficiency and perioperative transfusion is being prospectively audited and will inform a hospital-wide PBM program.

No conflict of interest to disclose.

358. Hemovigilance reports of complications of blood donation reported at a tertiary care centre in Karachi

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Aim:

The safety of the blood supply depends on the actions to protect both; blood transfusion recipient and the blood donor. Hemovigilance practice of learning of complications of blood donation and protecting them from such complications is the best way to minimize the risk to blood donor.

Method:

Comprehensive blood donor hemovigilance program was studied at Dr. Ishratul Ebad Khan Institute of blood diseases, Karachi from 2010 to 2015. Outlines of reported and communicated complications were collected after whole blood donation. Analysis was done by general logistic regression.

Result:

Complications after 30,000 Whole blood donation procedures calculated 1620 total (.54 per 1,000 donations). The majority of the complications were faint and pre-faint reaction with light headedness (58.6 %), Sore arm (24 %), Bruises and hematoma (14.4 %). Minor complications were Agitation/sweating (2 %) and arterial puncture (1 %). Markers of the complications were age, sex, race, weight, blood pressure and donation status. All associated independently after whole blood donation. Age and first-time status were associated with a significantly higher risk of complications with 18-22 years old at higher risk compared to 23 to 50 years old. First-time donor were at higher risk compared to repeat donor.

Conclusion:

The results of this study are helpful in identifying and understanding the promoter to complication of blood donation. Donor age and status were strong predictors of complications. The remedies and specific areas of care should be provided.

359. Knowledge, attitude and practice regarding the voluntary blood donation among young student population of Karachi

Nepal B¹

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Aim:

To assess the knowledge, attitude and practice regarding the voluntary blood donation among the young student population of Karachi so that an effective approach can be made regarding motivation enrolment of voluntary non remunerated blood donors in future in Pakistan

Method:

A cross sectional prospective study was conducted among 600 students from different universities and colleges of Karachi. A well-structured and pre-tested questionnaire, in English, was used to access the knowledge, attitudes and practices about voluntary blood donation. A scoring mechanism was used to understand overall knowledge level. Obtained data was analyzed.

Result:

The sample population consisted of 54% male and 46% female students in the age group of 18-28 years. Only 65 % of the students have heard about voluntary blood donation and 28 % of the students have given blood once in their lifetime and among them 19 % are blood donors at the moment. 42 % of the participants believed that there is a specific reason why they don't donate blood and 59 % believed that there is a risk involved for the donors, when donating blood. 80 % students wanted to promote voluntary blood donation. Fear and lack of awareness on blood donation are the reasons for not donating blood. Students gather information about voluntary blood donation from several sources mostly schools, colleges, family and friends.

Conclusion:

This study shows how increasing awareness and marketing through different ways can boost the culture of voluntary blood donation in society. Despite having the good knowledge about voluntary blood donation, very few students have donated blood. Blood donation education should begin at school level and courses should include blood donation drive. Student population can be motivated to participate in different ways. There is a dire need to mobilize the electronic media for educating our youth about voluntary blood donation due to its access to masses.

360. Distribution of blood donor in different age group in Kathmandu, Nepal

Nepal B¹

¹*Grande International Hospital, Kathmandu, Nepal*

Aim:

To explore the demographic distribution of the blood donor in different age group in the Kathmandu Nepal.

Method:

This is retrospective study conducted at Nepal Red Cross Society Central Blood Transfusion Service. Data from January 2013 to January 2017 were collected from donor management software .The data includes socio demographic data .Data has been process with SPSS version -17

Result:

During 4 years study period, total of 276,290 Blood donation happened from both mobile blood collection and in-house blood collection.

Out of 276,290 Collection, 48351 (17.5%) are from 18-24 age group; 106924 (38.7%) are from 25-31 age group 53324 (19.3%) are from 32-38 age group; 34536 (12.5%) are from group 39-45 age group; 23761 (8.6%) are from age group 46-52 and 9394 (3.4%) from age group above 53 respectively.

Conclusion:

The distribution of ABO blood group varies regionally and from one population to another. In Kathmandu, Nepal 18-38 years age group is the most common age group encountered donating blood. The data generated in the present study and several other studies of different geographical region of India will be useful to health planners and future health challenges in the region.

361. Haemovigilance reports of adverse blood donor reaction among VNRBD in tertiary care hospital in Nepal

Nepal B¹

¹*Grande International Hospital, Kathmandu, Nepal*

Aim:

To assess the adverse blood donor reaction among voluntary blood donors in tertiary care hospital in Kathmandu, Nepal

Methods:

This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive.

Results:

In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as, sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 8(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylaxis;11(10.49%), loss of consciousness; 3(2.85%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors.

Conclusion:

The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors is lower than in other studies. Donor age and donation status were strong possibilities of complications.

362. Frequency of reactive blood donors in a tertiary care hospital, Karachi, Pakistan

Nepal B¹

¹*Grande International Hospital, Kathmandu, Nepal*

Aims:

To find the frequency of different types of reactive healthy blood donors at a tertiary care hospital, Karachi, Pakistan.

Methods:

The retrospective observational study carried out on both male and female healthy blood donors. Data from complete blood screening from January 2013 to December 2014 were collected and frequency of various types of reactive blood donors was sorted out to get an actual picture. All the blood products were screened for HBV and HIV Using enzyme linked immunosorbent assay (Elisa plate washer version 3 and Elisa plate reader stat fax 3200). HCV screening was performed on Architect 2000 SR Chemiluminescent micro plate immune assay (CMIA). Malarial parasite tested by making thick and thin smear seen under microscope. Syphilis was tested by ICT method.

Results:

A total number of 6996 healthy donors were received and about 624 were found to have blood screening positive in various combination. The highest numbers of isolates was HbsAg reactive 214, HCV 213, VDRL 170, HIV 26 and 1 case of malarial parasite. More prevalent in male population. In this study there were seven donors found with HCV –VDRL co infection and five co-infected with HCV and HbsAg two donors with HIV and HbsAg infected and two donors were HbsAg and VDRL reactive.

Conclusion:

This study supports that HBV, HIV and syphilis prevalence is high and HIV prevalence is low in healthy blood donors.

363. Major blood groups and A₂ sub-group in mixed Pakistani population of Karachi.

Nepal B¹

¹*Grande International Hospital, Kathmandu, Nepal*

Aim:

To observe the frequency of major blood groups and sub-group A₂ in a mixed Pakistani population in the catchment area of Dow international medical college, Karachi.

Method:

536 healthy unrelated donors and their patients for whom they donated blood, of both sexes were selected for four months. Venous samples were taken and forward and reverse grouping were done by tube method in the blood bank of DDRRL affiliated with Dow international medical college and its affiliated Ojha hospital.

Result:

The dominant group was "O" n 111(40 %) and B group was the second largest n 149 (39 %). The third largest was A group n 72 (20 %) and least was n 13 (4%). From A group, 20 were A₂ group. This constituted 3 % of all samples and 18 % of A group individuals.

Conclusion:

The blood group frequency of a mixed Pakistani population had differences with the studies done in other countries and for the first time, the frequency of A₂ sub-group was estimated in mixed Pakistani population.

364. Reasons for blood donor deferral among voluntary blood donors in a tertiary care hospital, Nepal

Nepal B¹

¹*Grande International Hospital, Kathmandu, Nepal*

Aim:

To assess the Reasons for blood donor deferral among voluntary blood donors in a tertiary care hospital in Kathmandu, Nepal

Methods:

This is the retrospective study carried out among voluntary blood donors at Grande International Hospital, a tertiary care hospital in Kathmandu, Nepal from January 2013 to January 2015.

Results:

The data were collected from previous records of the blood donor history forms. From a total of 8,550 blood donations, 302(3.53%) blood donor were deferred due to various reasons. Among all the deferred blood donors 189(62.58%) were female where as 113(37.42%) were male. Furthermore, 289(95.69%) were temporarily deferred and 13(4.31%) were permanently deferred. The mean age of deferred blood donor was 35 years.

Out of total blood donor deferral; 101(33.48%) donor were rejected because of bed side hypertension (i.e. blood pressure- systolic > 140 and diastolic >90 mm hg) which was followed by anaemia(i.e. haemoglobin < 12 gm/dl) 94(31.12%), vaccinations history 43(14.23%), hypotension (i.e. Systolic < 90mm hg and diastolic < 60 mm hg) 35(11.58%), dental examination 10(3.32%) and medication history 6(1.98%). Permanent deferral namely, risk factor involving transfusion transmitted infections and chronic disease were 5(1.65%) and 8(2.64%) respectively. The prime cause of permanent deferral was risk factor involving transfusion transmitted infections while the temporary deferral was bed side hypertension. Gender wise, the leading cause of donor deferral in male was bed side hypertension and anaemia was the major cause in female.

Conclusion:

The findings of the survey aid to evaluate the significant causes of blood donor deferral. This study suggests that the restrictive criteria can be used for blood donor selection. This will in turn increase the blood supply of tertiary care hospital.

365. Strategies to improve supply of phenotyped red cells.

Parsons K¹

¹*Australian Red Cross Blood Service, Alexandria, Australia*

Aim:

To identify strategies to improve supply of phenotype red cells nationally, in addition to implementation of an automated phenotyping platform.

Method:

The procedures for the supply of phenotyped red cells were reviewed and process-mapped to identify current procedures nationally, highlighting regional variations. An ideal process map was developed identifying opportunities for improvement. New deferral codes were created, documents were updated and staff training was provided. Communications were provided to customers.

Results:

A standardised national process was adopted. A procedure was created to enable phenotyped donors to be called for specific blood orders. Education of customers regarding appropriate order lead times and ordering, based on the ANZSBT guidelines and the NPAAC requirements, was provided via a written communication, customer forums and on a case by case basis. Reports with required phenotyping numbers to meet desired inventory levels were created, though an automated phenotyping platform is required for full implementation. In the interim manual phenotyping targets for each state were adopted. A code was created for use on rare or uncommon phenotyped donors, to identify them as preferred whole blood donors.

Conclusion:

An improvement in the provision of phenotyped red cells measured by DIFOT (delivery in full on time) has been seen both in NSW and nationally as a result of these changes.

366. The importance of laboratory contingency planning.

Parsons K¹

¹*Australian Red Cross Blood Service, Alexandria, Australia*

Aim:

To identify possible scenarios that may disrupt routine processes within the Blood Service Red Cell Reference laboratories. For each scenario, contingency procedures are documented and are in place to ensure there is no loss of critical functions.

Method:

All Red Cell Reference laboratory processes were identified and analysed to determine if critical or non-critical, the time allowable without the process before there is an impact on the blood supply or patient care. For each critical process, resources required to keep the process operating were identified, including facilities, people, information systems, suppliers, specialised equipment and vital records.

Result:

Being a national Red Cell Reference team, having laboratories in four separate sites and states, it was determined that sufficient facilities, equipment and staffing are already in place to offer contingency if any of the laboratories, sites, or staff at a particular site are not available. Alternate reagents, equipment and testing methods/processes are already available for all critical testing performed, and alternate procedures are available for when information systems and records are not available.

Conclusion:

The Blood Service Red Cell Reference laboratories are sufficiently equipped and have plans in place to maintain critical functions in the event of an unplanned event that may disrupt routine processes. Having such arrangements in place ensures continuity of care for both donors and patients.

372. Development of a novel whole blood model to assess transfusion related modulation of inflammasome activation

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Background and Aim:

Transfusion has been reported to modulate recipient immune responses, however the mechanisms are largely unknown. Interleukin-1 β (IL-1 β) is an important mediator of the inflammatory response and is involved in cell proliferation, differentiation and apoptosis. Inflammasomes are important innate immune multiproteins that when activated cause a proteolytic cascade to form mature IL-1 β , which is regulated by caspase-1. Using an *in-vitro* transfusion model, we have previously reported that exposure to blood components modifies production of IL-1 β ¹ suggesting that inflammasome activation may be one mechanism associated with transfusion-related immune modulation. We aimed to develop a novel whole blood method for assessing inflammasome activation (detection of activated caspase-1) that can be used to investigate whether blood transfusion modulates this important part of the innate immune response.

Methods:

Whole blood from healthy volunteers was collected (n=6) and left untreated, or stimulated with lipopolysaccharide (LPS, 4 h) or LPS (4 h) + ATP (additional 15 mins) at 37°C (5% CO₂). Blood was then harvested, plasma removed and cells stained with CD45-PerCP and CD14-V500. Cells were washed and red cells lysed. FAM-FLICA caspase assay (Immunochemistry Technologies, Bloomington, USA) was then used to assess inflammasome activation. The proportion of monocytes (CD14⁺CD45⁺ cells) with activated caspase-1 was assessed (paired T-test).

Results:

We report for the first time, detection of inflammasome activation in a whole blood model. Inflammasome activation was detected in untreated cells (mean 8.60, SD 1.44), following LPS (mean 23.82, SD 10.96) and LPS+ATP treatment (mean 30.73, SD 15.30). LPS alone and LPS+ATP significantly increased inflammasome activation compared to untreated cells (P=0.008, P=0.007 respectively).

Conclusion:

We have developed a novel method for detecting inflammasome activation without the need for cell isolation, which will now be applied in our established *in-vitro* transfusion model to provide further evidence that blood transfusion modulates inflammasome activation.

373. Blood bank management of a patient with anti-JMH: a case report

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Background:

JMH is a blood group consisting of 6 high-prevalence antigens, which are located on the Semaphorin 7A protein. The protein plays an important role in the immune and nervous systems. Three low frequency phenotypes have been found: JMH-weak, JMH-negative, and JMH-variant. JMH-variant is caused by a missense mutation in the SEMA7A gene on chromosome 15. JMH-weak and JMH-negative are usually acquired, and can be transient, during which anti-JMH may develop. Weak and negative phenotypes are usually found in elderly patients, and less commonly inherited. Anti-JMH present in patients with acquired JMH-negative, or JMH-variant phenotypes usually have autoimmune characteristics (i.e. DAT positive due to existing expression of JMH on the patient's red cells), but are not considered clinically significant, and rarely causes reduced red cell survival.

Case Presentation:

Mr. AK, 88-year-old European patient, was scheduled for a hip replacement surgery. A group and hold sample was received for pre-transfusion testing. The patient had no antibodies present in previous group and hold tests. The new group and hold however showed the presence of an antibody consistently positive in all panels tested, but non-reactive using ficin-treated cells. Following extensive investigation by the local reference laboratory, anti-JMH was identified. Based on the findings, 4 red cell units, matched to the patient's phenotype for ABO, K, Fy, Jk and S, were provided, under the discretion of the Transfusion Medicine Specialist. 3 units were transfused post-surgery. No signs of haemolysis or transfusion reactions were noted.

Conclusion:

Anti-JMH is a rare antibody that is not considered clinically significant. Due to the rarity of JMH-negative/variant phenotypes, compatible red cell units are not usually available, and least incompatible units are given. While it rarely causes reduced red cell survival, haemolysis can still occur. Therefore it is recommended to prevent transfusion if possible, and maintain haemoglobin levels using other measures.

374. National Patient Blood Management Implementation – the next 4 years

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Aim:

To describe how the next National Patient Blood Management Implementation Strategy 2017-2021 takes a patient-centred approach intended to optimise clinical outcomes and improve patient safety

Method:

Patient Blood Management (PBM) improves patient outcomes by ensuring that the focus of the patient's medical and surgical management is on optimising and conserving the patient's own blood. PBM is not an intervention or an alternative to allogeneic blood transfusion; it is sound evidence-based clinical practice.

The operational and cultural change required to implement best practice clinical outcomes at a health provider level are significant and sometimes require complex changes in business process and clinical practice. There are also a range of wider environmental challenges (such as organisation buy in, funding pressures and clinical champions) confronting jurisdictions and health service organisations seeking to implement the change.

Results:

Since the launch of the PBM Guidelines and initial implementation strategy, Australia has seen a significant reduction in the use of red blood cells. The implementation of the Standard (a dedicated hospital accreditation standard for Blood and Blood Products and the revised Blood Management Standard) has also contributed to this decline in the use of red blood cells. This reduction in use would not have occurred however, without the concerted effort of jurisdictional programs, clinical PBM champions and a willingness by healthcare professionals to adopt a patient focus rather than a product focus and using blood and blood products more appropriately and safely.

Conclusion:

The core element of the next strategy is to facilitate activities and development of materials at a national level that support implementation at a health provider level. The six main elements covered in the strategy include:

- PBM Guidelines
- PBM tools and resources
- Education and Training
- Promotion and communication
- Data

Research and development

375. Successful interdisciplinary collaboration - a case study

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Aim/Background:

Safe and effective patient care often demands an interdisciplinary collaborative approach.

We describe such a case in our hospital of a 20 year old Female, low risk pregnancy; where prompt recognition by the laboratory of an undisclosed large Foetal Maternal Haemorrhage (FMH); combined with foetal monitoring triggered an emergency caesarean section resulting in good outcomes for both mother and neonate.

Method:

29/07/16 induction, Foleys catheter inserted.

30/07/16

15:00, Labour augmented with artificial rupture of membranes (ARM) and IV oxytocin, continuous foetal monitoring commenced.

15:20 Group & Hold sample collected. Tests performed by column agglutination technology.

16:30 Repeat sample to confirm indeterminate maternal ABO group.

17:15 Kleihauer-betke (KB) screen performed to confirm presence of foetal cells. High count confirmed by Flow Cytometry.

18:15 Non reassuring changes on CTG.

19:00 Obstetric Neonatal Emergency Team activated (ONET) Cat 2 emergency caesarean section performed.

Results:

Indeterminate ABO RhD group on maternal sample, raised suspicion of artefact, recent transfusion or a dual cell population.

Repeat sample confirmed indeterminate group - recent transfusion, artefact excluded.

KB screen demonstrated 4250 foetal cells, suggestive of an 85 ml FMH, confirmed by Flow Cytometry.

Foetal distress detected by decelerations on CTG combined with knowledge of large FMH, prompted an emergency caesarean Section.

A slightly pale but otherwise healthy neonate delivered.

Intravenous Rhophylac prophylaxis administered post-delivery to mother.

Mother and neonate discharged 5 days post-delivery in good health.

Conclusion

FMH, the leakage of foetal cells into maternal circulation, takes place in normal pregnancies as well as when there are obstetric or trauma related complications. The majority of bleeds are small, < 1 ml; in contrast a large bleed >30 ml, is a rare event; symptoms of FMH are often subtle and nonspecific making it difficult to identify.

A large undisclosed FMH can be a life threatening entity and considered a medical emergency demanding an interdisciplinary collaborative approach to aid in best possible patient outcomes.

376. Blood transfusion in older adults – how will ageing impact sustainability of the blood supply?

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Aims:

Our study examined blood use by older adults admitted through emergency departments in the Sunshine Coast region, and the implications for blood demand and patient blood management.

Methods:

Data were obtained from a range of sources, including: Australian Bureau of Statistics historic population data and projections; Sunshine Coast Hospital and Health Service (SCHHS) Emergency Department (ED) information system records; SCHHS patient admission system records; Pathology Queensland transfusion records.

Regional population, ED presentation, hospital admission and blood use data were separated by patient gender and stratified into five-year age bands. Data were analysed to determine the rates of red cell use per 1,000 population and per 1,000 ED presentations. The timing of transfusions relative to ED presentations was determined.

Results:

The population aged 65 years or above in the Sunshine Coast region is 30% higher than the Australian average. Male life expectancy is increasing at a faster rate than female life expectancy in Australia.

Across the SCHHS, 60% of blood was used by patients aged 65 years and above. The rate of blood use in older males was higher than in older females. Almost 30% of blood used across the SCHHS was transfused with 24 hours of an admission through ED.

Conclusions:

The over-65 age group is the fastest growing cohort in Australia. This combined with high rates of blood use in older adults has significant implications for blood supply planning. Further research is needed to explore the high rate of blood use proximal to ED presentations, and its implications for patient blood management.

377. An audit of clinical indications of cryoprecipitate usage in SA metropolitan hospitals

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Aim:

There has been a steady increase in use of cryoprecipitate in SA metropolitan hospitals. The aim of the study was to review patterns and indications for use of cryoprecipitate including wastage in SA Public hospitals.

Method: A linked database containing clinical, hospital morbidity and transfusion data within 7 major SA public metropolitan hospitals was used and reviewed for 2014-2016 FY. For the purposes of this audit, all usage was converted to standard adult and paediatric doses. Standard adult dose of cryoprecipitate is 10 units of whole blood cryoprecipitate and 5 units of apheresis cryoprecipitate. Pre-transfusion fibrinogen levels and cryoprecipitate discards were also evaluated.

Results:

A total of 1408 doses, 1271 adult and 137 paediatric doses for 722 admissions were analysed. The clinical scenarios with highest use of cryoprecipitate doses were cardiac surgery (310, 22%), haematology/oncology (220, 15.6%), critical care (150, 10.6%), liver transplant (136, 9.7%) and other surgery (132, 9.4%). Among the paediatric doses, the majority were used by neonates (32.8%) and patients undergoing neurosurgery (21.1%). A total of 895 units (10.1%, 172 doses approximately) of cryoprecipitate were discarded during the review period of which 119 doses were discarded due to expiry of thawed units. Pre-transfusion fibrinogen were available for 1073 transfusion episodes and the median pre-transfusion fibrinogen was 1.4 (IQR1-2).

Conclusion:

Early cryoprecipitate for major haemorrhage and cardiac surgery including thromboelastometry guided cryoprecipitate transfusion has led to increase in use of cryoprecipitate in recent times. Measures are required to manage the high wastage rate of cryoprecipitate due to expiry of thawed units.

378. Analysis of O RhD negative red cells in South Australian metropolitan hospitals

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Aim:

Aim of the study was to understand the use of O RhD negative red cells (RC) at three different metropolitan hospitals in South Australia.

Method: An audit of O RhD negative red cells (RC) use was conducted for a three month period (April-June 2016). All RC transfusion within the review period were classified into 4 main categories- O negative patients, emergency, specific requirements and avoid expiry.

Results:

A total of 788 O RhD negative RC units were included in the audit. In total 35.2% (277) were used in O RhD negative patients, 14.3% (113) for emergency use, 10.5% (83) for specific requirements, and 40% (315) to avoid expiry. Table 1 show the distribution of O RhD use by hospital. Of the 315 units to avoid expiry, 94.3% (297) were transfused to O RhD positive patients. Overall 78% of the O RhD negative RCs used to avoid expiry were units transferred from other hospitals or laboratories with 31.9% , 81.5% and 93.5% at Hospital 1,2 and 3 respectively.

	Hospital 1	Hospital 2	Hospital 3
1	45 (33.6%)	15 (27.3%)	217 (36.2%)
2	10 (7.5%)	11 (20%)	92 (15.4%)
3	7 (5.2%)	2 (3.6%)	74 (12.4%)
4	72 (53.7%)	27 (49.1%)	216 (36.1%)

Conclusion:

A high proportion of O RhD RC units were used to avoid expiry at all three sites. With BloodMove program in place in SA, metropolitan laboratories have to manage near expiry RC. This audit will help us to take initiatives such as evaluation of inventory holdings both at regional and metropolitan laboratories, review hospital laboratory /transfers and other measures to manage appropriate O negative red cell use.

379. Does the red blood cell phenotype influence an anti-D donor's ability to develop anti-D antibodies?

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Cases of haemolytic disease of the foetus and newborn have declined significantly since the introduction of successful routine administration of prophylactic anti-D immunoglobulin (Ig) to susceptible RhD-negative pregnant women. The Blood Service conducts an Anti-D Program to actively immunise selected RhD-negative blood donors with small volumes of RhD-positive red blood cells (RBCs) to stimulate anti-D Ig production. Approximately 50% of primarily immunised donors develop serum anti-D Ig concentrations >1 IU/mL and are assigned a 'Responder' profile. We have previously sought to examine the donor genetic factors associated with the donor responder profile (Tan et al., 2015). Here, we hypothesise that the RhD-positive RBCs phenotype could also influence the donor responder profile. In a survey of 171 anti-D donors where the immunisation record was collected, we determined that 62% of n=34 female anti-D donors and 47% of n=137 male anti-D donors were Responder donors (p value = 0.038). We found that Non-Responder donors were unlikely to develop anti-D antibodies with additional booster injections. We further examined a subset of 114 anti-D donors who were immunised with RhD-positive RBCs belonging to only one haplotype: either R0 (Dce), R1 (DCe), R2 (DcE) or RZ (DCE). We found that 57% of donors immunised with R2 only and 54% of donors immunised with R1 only RhD-positive RBCs developed anti-D Ig, as opposed to donors immunised with R0 only (42%) or RZ only (33%). Responder anti-D donors also developed antibodies to RBC antigens to which we did not match when selecting RBCs for immunisation. Further data collection and analysis will allow us to determine if the red blood cell phenotype can influence an anti-D donor's ability to develop anti-D antibodies.

Tan, J.C.G., Armstrong, N.J., Yuan, F.F., Flower, R.L., Dyer, W.B., 2015. Identification of genetic polymorphisms that predict responder/non-responder profiles to the RhD antigen. *Molecular Immunology* 68, 628-633.

380. A implementation of a major haemorrhage starter pack to minimize massive transfusion protocol activations in the Emergency Department

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Aim:

A previous audit has shown that over half of all MTP activations by the Emergency Department (ED) at our tertiary-care institution were not used. In absence of a blood fridge in the ED, a 'Major Haemorrhage Starter Pack' (MHSP) consisting of 4 units of O Neg red cells (RC) was implemented in April 2017. The aim of the starter pack was to minimize the pre-emptive activation of MTP in particular for pre-admission of patients with suspected haemorrhage and also to reduce wastage. The aim of the study was to examine the number of MTP activations and usage of MHSP 2 months post implementation.

Methods:

All MTP activations from ED for a 2 month period (19th April - 22nd June 2017) were analysed. Data collected comprised the total number of MHSP used, MTP activations, whether the packs were unused, partially used or completely used and the quantity and type of blood products transfused.

Results:

A total of 13 MTP activations and 17 MHSP were used in ED during the study period. A median of 2 (IQR 1-4) RC units were used by the patients using MHSP. MTP was not activated in 50% of patients using MHSP. Compared to the same period in the previous year there was 43.4% reduction (23 vs 13) in MTP activations. Of the 13 MTPs activated, seven (53.8%) were returned unused. Five of those seven unused activation were for trauma patients and post MHSP use.

Conclusion:

Within two months of implementation, a significant reduction in MTP activations has been demonstrated. Although the number of MTP activations has decreased in ED, there has been no change in the rate of unused MTP activations. This most likely due to the ED policy of MTP activation post MHSP use. Use of the clinical ABC score and review of post MHSP use MTP activation policy may also help in optimizing the need for MTP activation in ED for trauma patients.

381. Challenges of introducing a new labelling standard to the Australian Blood Supply

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The Australian Red Cross Blood Service is entrusted with the supply of Australia's blood products. Every year 1.3 million blood donations are collected, tested and manufactured into blood components, then delivered to hundreds of healthcare providers around the country. Each blood component is provided with a specific label that contains barcoded information for use by health care providers to manage blood inventory.

The Blood Service has restarted work to implement the Information Standard for Blood and Transplant (ISBT128) in accordance with the National Blood Authority's barcode specifications paper 'Barcode Specifications for Blood and Blood Products Funded under the National Blood Arrangements'. This will be achieved by supplying blood components with an ISBT128 'transition' label to health providers from June 2018.

The ISBT128 standard will provide the highest levels of accuracy, safety and efficiency for the benefit of patients and healthcare providers by delivering internationally recognised standards for barcoding of fresh blood components. Changes to the component release label and associated data structures, such as donation identification numbers and component codes, while seemingly simple, will mean significant change through the Blood Service and to external stakeholders.

Change management and communication is critical to the success of the implementation of ISBT128 both internally within the Blood Service and externally to customers. The changes involved with ISBT128 are technical and multi-faceted, requiring modifications to information systems, equipment and processes.

This presentation will highlight the challenges the Blood Service has experienced and the approach in trying to adequately prepare Blood Service staff and health providers receiving and managing blood components for such a major change.

Australian Red Cross Blood Service is funded by Australian Government's

382. Red blood cell wastage in Victoria – where are we at?

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Background:

In July 2014, a red blood cell (RBC) wastage reduction project commenced to assist Victorian health services to reduce RBC wastage to specified targets as set by National Blood Authority (NBA). Most recent targets for 2017/18 are set at 1.5 to 2.5%.

Method:

The project implemented strategies (Table 1) to help health services reduce RBC wastage and these continue to be a central part of the ongoing success.

Table 1. Red cell wastage reduction project strategies

- Regular reporting of up-to-date wastage to those tracking above set targets
- Monthly wastage reports generated for requesting pathology laboratories
- Hub and spoke modelling in place across Victoria and operating effectively
- Site visits to new and existing RBC customers to discuss project
- Ongoing inventory management discussions based on wastage results
- Tools for blood fridge maintenance
- Facilitated blood fridge data sharing between pathology sites
- Audit tool to calculate the crossmatch/transfusion ratio
- Annual Victorian wastage summit
- 'STOP the waste' festive campaign 2015/16 and 2016/17
- Presentations at forums and conferences showcasing the success of the project

Results:

Nationally, over 1.9 million RBC units were issued between July 2014 and May 2017; with 29% (547,584) issued to Victoria. At the commencement of the project, in July 2014 nationally 2755 RBCs were discarded, with Victoria discarding 931 units. In May 2017 1285 RBCs were discarded nationally, with Victoria discarding 378 units. Overall Victorian RBC waste continues to decrease, the period between June 2016 to April 2017 revealed Victorian RBC wastage to be consistently below the national RBC wastage figure.

Conclusion:

A multi-faceted approach to reduce RBC wastage has successfully achieved most targets set for health services, reducing Victorian wastage from 5.6% to an average of 2.1% in 2017. While strategies are in place and working, ongoing collaboration with health/pathology services is crucial to continued success of the project.

383. Combining UVC pathogen inactivation and cryopreservation: A novel approach to platelet storage.

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Aim: Pathogen inactivation (PI) treatment of platelets prior to cryopreservation may allow for the extension of shelf-life following thawing and improve product safety. This study aimed to determine the effect of combining PI-treatment and cryopreservation on platelet quality.

Methods: ABO-matched buffy-coat derived platelets were pooled and split to form matched pairs (n=6). One unit remained untreated, and the other was treated with the THERAFLEX UV-Platelet system according to manufacturer's instructions (MacoPharma). The units were frozen in 5-6% DMSO at -80°C according to published methods. Platelets were thawed and resuspended in plasma. Activation markers were analysed by flow cytometry. Dynamic light scattering (ThromboLUX) was used to assess the particle composition within the components. Post-thaw platelet function was analysed using thromboelastography (TEG). Statistical comparisons were performed using a paired two-sided t test and a p-value <0.05 was considered significant (*).

Results: PI-treated platelets had a lower *in vitro* recovery following cryopreservation. While the proportion of platelets expressing P-selectin (CD62P) was lower in PI-treated platelets, a higher proportion of platelets expressed phosphatidylserine (Annexin V). PI-treated platelet components contained a higher proportion of microparticles than untreated controls. The time to clot formation of PI-treated platelets (R-time) was similar to untreated controls, however, the strength of the clot formed was reduced (MA).

	Untreated	PI-treated
Recovery (%)	72 ± 3	49 ± 3*
CD62P (% positive)	19 ± 5	12 ± 4*
Annexin V (% positive)	60 ± 7	74 ± 5*
ThromboLUX (% microparticles)	24 ± 9	34 ± 7*
TEG R-time (minutes)	3.4 ± 0.3	3.4 ± 0.2
TEG MA (mm)	61.6 ± 5.5	50.5 ± 3.4*

Conclusion: PI-treatment of platelets prior to cryopreservation alters the expression of activation markers, increases the microparticle release, and affects platelet function. This data suggests that combining PI-treatment and cryopreservation may reduce product efficacy.

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

384. Pathogen inactivation doesn't impact the glycosylation pattern of cold-stored platelets but reduces glycans following cryopreservation.

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Aim:

Platelets for transfusion are conventionally stored at room-temperature (20-24°C), which limits their shelf-life to 5 days. Modifications to storage such as cold storage (2-6°C), cryopreservation (-80°C), or pathogen inactivation (PI) of platelets may facilitate extension of the shelf-life and improve product safety. However, these treatments differentially affect aspects of platelet quality. Desialylation of platelet glycoproteins, which is involved in mediating platelet clearance following transfusion, is thought to be accelerated by these treatments when used individually, yet the effect of combined treatment remains unknown. As such, the impact of PI on the glycosylation patterns of platelets stored under alternate conditions is yet to be explored, and was the focus of this study.

Methods:

Buffy-coat derived platelets were pooled and split to form matched pairs. One unit remained untreated and the other was treated with UVC light using the THERAFLEX UV-Platelets system according to manufacturer's instructions (MacoPharma). Platelets were stored at room-temperature (RT), refrigerated (cold), or cryopreserved in 5-6% dimethylsulfoxide at -80°C. The exposure of specific glycan residues was assessed by flow cytometry using the following lectins: *Sambucus nigra* (SNA) for sialic acid, *Ricinus communis* agglutinin (RCA) for galactose and succinylated wheat germ agglutinin (sWGA) for N-acetyl-D-glucosamine (βGlcNAc).

Results:

The surface binding of SNA (sialic acid) on RT and cold platelets decreased by two-fold over the storage period ($p < 0.0001$) but all treatment groups were affected similarly ($p = 0.2986$). Surface binding of RCA (galactose) and sWGA (βGlcNAc) on RT and cold platelets remained stable over the storage period, and was not affected by PI treatment ($p = 0.8853$ and $p = 0.9971$, respectively). In contrast, there was an approximate 2-3-fold reduction in the binding of all lectins to cryopreserved platelets, compared to room-temperature stored platelets ($p < 0.0001$), and PI treatment induced a further reduction in the binding of all lectins ($p < 0.001$).

Conclusion:

These data suggest that PI-treatment of cold stored platelets may allow for an extended shelf-life and improved product safety without adversely affecting the clearance rates following transfusion. Further work is required to understand the effect of PI treatment of cryopreserved platelets on clearance.

Conflict of interest:

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

385. Patterns of fresh frozen plasma use in a Queensland regional hospital

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Aim:

To establish the patterns of FFP usage in a regional Queensland hospital, and determine if its use is aligned with current clinical practice guidelines

Methods:

A retrospective audit of 67 individual FFP transfusion episodes at Nambour General Hospital from July 2016 to March 2017 were identified via the laboratory information system. Data was abstracted from the electronic medical record to determine i) prescribing specialty, ii) patient bleeding status, iii) indication for use, iv) dose prescribed, v) INR pre- and post-transfusion

Results:

FFP prescribing aligned with clinical guidelines in 61.2% of patients overall. The emergency department and the intensive care unit most commonly prescribed FFP, with relatively high rates of aligned prescribing (76.2%). Lower rates were seen in surgical (44.4%) and medical (33.3%) units. The most common indications for FFP use was activation of the massive transfusion protocol (31.3%), liver disease (26.9%), and warfarin reversal (16.4%). FFP prescribed for major bleeding was aligned with guidelines in 88.9% of patients, compared to 27.8% in patients who were not actively bleeding. Dosing was aligned with guidelines (10-15mL/Kg) in 53.4% of transfusions, with frequent underdosing. A greater mean change in INR was seen in aligned transfusions.

Conclusions:

This audit confirms the high rate of nonaligned FFP use, widely published in Australian and International literature, and identifies the specialties and clinical scenarios this is more likely to occur. This will allow specialty specific local guidelines and targeted education to be developed to improve appropriateness of FFP use.

386. Immunoglobulin therapy in CLL: the Sunshine Coast experience

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Aim:

To describe management and outcomes of patients receiving immune replacement therapy (IRT) for hypogammaglobulinaemia secondary to Chronic Lymphocytic Leukemia (CLL)

Method:

Retrospective study of prescribing practices and treatment outcomes in patients treated at Nambour General Hospital, Queensland receiving either intravenous or subcutaneous immunoglobulin (IVIg or SCIg)

Results:

Seventeen consecutive patients with CLL were initiated on IRT January 2012 to September 2015, median followup since commencement of IRT 30 months (range 2-93). Patients had a median age of 69 years, and 1 prior line of chemotherapy, including alkylators (82%), Rituximab (76%), Fludarabine (59%), or no prior treatment (18%). All patients initially received IVIg 0.4g/Kg monthly, with 3 transitioning to SCIg.

In the 12 month period pre-and post- commencement of IRT, the median number of hospital admissions and hospital days with infections were 1 vs 0 and 4 vs 0, respectively. Baseline median IgG levels were 3.7g/L, increasing to 7.6g/L after 3 months of IRT, with monitoring performed on 0-8 occasions (median 3) in the first 12 months of IRT.

IRT was initiated during a hospital admission in 29% of patients, and from the outpatient clinic in the remaining 71%. Attempts at IRT discontinuation was made in 9 patients (53%): 4 remain off treatment, 3 recommenced therapy after a median of 25 months, and 2 deaths occurred from unrelated causes.

Conclusions:

IRT normalises IgG levels in CLL patients with secondary hypogammaglobulinemia, however has a modest effect on severe infections. The majority of patients were commenced on IRT in the outpatient setting, potentially representing an opportunity for increased SCIg utilisation. Treatment was able to be either interrupted or discontinued for prolonged periods in around half of patients. Inconsistent monitoring of levels was observed.

387. Management of a repeat case of severe fetal anaemia due to HDFN – Auckland Blood Bank

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Background:

Fetal anaemia due to red cell alloimmunisation can be associated with significant neonatal morbidity and mortality. However, since the utilisation of intra-uterine transfusions (IUTs), survival rates have improved significantly in comparison to other causes of fetal anaemia.

Case:

Mrs. R is a 36 year old who has had 13 pregnancies from the year 2000, comprising of 4 miscarriages and 9 live births, and is currently pregnant. We do not know the details of her miscarriages, but given her case, we suspect that they were on the basis of HDFN. In total, she has developed 4 alloantibodies (D, Fya, Jkb, and an unidentified). She has no significant comorbidities or hereditary diseases. In total Mrs. R has had 13 IUTs in her last four pregnancies, including two during this current pregnancy, with the potential for more in her current pregnancy. She presents for assessment at 24 weeks each time but the fetal anaemia has not been worsening, despite the father being homozygous for D, against which the mother has a titre of 512.

Discussion:

Mrs. R has had the most IUTs of any women in NZ. This case is complex because of her rural location and religious beliefs, which do not allow for the use of contraception. Although she has the potential to develop more antibodies with future pregnancies, this is somewhat tempered by her having a single partner. To our knowledge there are only two other cases in NZ of women who have had further pregnancies after requiring an IUT; in both cases their subsequent pregnancy was with a new partner and did not require an IUT, which makes our case unique. The stability of her titres and the lack of progression of fetal anaemia in subsequent pregnancies may be instructive in counselling other patients in similar circumstances.

388. Management of iron deficiency anaemia in a regional Australian hospital.

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Aim:

To evaluate the management of patients with iron deficiency anaemia and determine compliance with the National Blood Authority's (NBA) Patient Blood Management Guidelines.

Methods: A retrospective audit was conducted by reviewing the electronic medical records of all patients admitted to a regional Australian hospital with a diagnosis of iron deficiency anaemia, over the six-month period from October 2016 to March 2017. Clinical and laboratory data was collected and examined to determine if the patient management was consistent with the NBA's Patient Blood Management Guidelines, in particular the single unit transfusion guide.

Results:

92 stable, normovolaemic patients with iron deficiency anaemia were included in the audit. 47 patients (51%) received an initial red cell transfusion, of whom 9 patients (20%) received a single unit, 34 patients (72%) received two-units and 4 patients (8%) received three-units. Evidence that the single unit transfusion guide was followed was found in only 4 patients (8%). 22 patients (24%) were aged under 65 years, 11 of whom (50%) were transfused, although end-organ compromise justifying red cell transfusion was documented in only one patient. 70 patients (76%) were aged over 65 years, 36 of whom (51%) were transfused, with end-organ compromise being documented in 13 patients. 7 of the 8 patients (88%) with haemoglobin less than 60 g/L were transfused; 22 of the 25 patients (88%) with haemoglobin 60-80g/L were transfused; and 18 of the 60 patients (30%) with haemoglobin greater than 80 g/L were transfused. Iron therapy (oral or intravenous) was prescribed in 73% of those aged under 65 years and 80% of those aged 65 years or over.

Conclusions:

There is a significant discrepancy between the management of patients with iron deficiency anaemia at our hospital and the NBA's Patient Blood Management Guidelines. Improving awareness of the single unit transfusion policy, including the indications for red cell transfusion in patients with iron deficiency anaemia, will reduce patient's exposure to allogeneic blood and its associated risks.

389. 'Kaizen' applied to adsorption investigations, New Zealand Blood Service, Christchurch, New Zealand.

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Background and Aim:

Adsorptions are resource-hungry, but useful, immunohaematology tests which, by removing pan-reactive auto-antibody, can help reveal underlying allo-antibody. In the spirit of 'Kaizen' ('continuous improvement' in Japanese), we examined numbers of procedures, adherence to guidelines¹⁻³, and allo-antibody yield.

Methods:

Retrospective audit of adsorptions at (or requested by) NZBS, Christchurch between 01/01/2007-31/12/2016. Standard techniques were used for adsorptions.

Results:

281 adsorptions (117 auto-/164 allo-) on samples from 134 patients were studied. All samples were pan-reactive on antibody screening/identification. 101/117 (86%) auto-adsorptions were appropriate (pan-reactive + no recent [3m] h/o transfusion/pregnancy + Hb \geq 70g/L). 146/164 (89%) allo-adsorptions were appropriate (pan-reactive + h/o recent (3m) transfusion/pregnancy +/- Hb \leq 70g/L). 119/281(42%) of all adsorptions were on samples from patients *without a h/o prior transfusion/pregnancy* including 40 (14%) on samples from *males without h/o prior transfusions*. Since November 2011 prophylactic antigen-matched (PAM) RBC transfusions have been used (to the extent possible) for certain patients reducing alloimmunization risk even in transfused patients and thus, hopefully, reducing the necessity for adsorptions. The 'pre-PAM' period (58 months) saw 196/281 (70%) and the 'post-PAM' period (62 months), 85/281 (30%) procedures performed respectively. An underlying allo-antibody was detected following 22(19%) and 79(48%) of the 117 and 164 auto- and allo-adsorptions respectively; in 18(17%) and 83(48%) of males and females respectively; and, finally, in 89(88%) and 12(12%) of those with/without prior transfusion/pregnancy respectively.

Conclusions:

Up to 42%, but at least 14%, of 281 adsorptions may have been avoidable because there was, likely, no prior allo-immunising event. 101/281 adsorptions (36%) revealed an allo-antibody but it is unclear why allo-adsorptions were more efficient than auto-adsorptions. Not surprisingly, more allo-antibodies were found in females and in those with prior allo-immunising events. More attention to allo-immunization history may reduce adsorption procedures further. The choice of auto-/allo-adsorption was appropriate in most but, in 16/117(14%) and 18/164(11%) of auto- and allo-adsorptions respectively, the alternative may have been better.

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390. SCIg home-based immunoglobulin replacement therapy is a cost-effective treatment for Australian primary immunodeficiency patients

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Background:

Immunoglobulin replacement therapy (IRT) accounted for 48% of the 2014-15 total Australian blood budget. Hospital-based intravenous immunoglobulin (IVIg) is the predominant form of IRT in Australia, with only 1.52% of patients using subcutaneous immunoglobulin (SCIg) at home. In contrast, approximately 50% of patients in the USA and Europe are using SCIg.

Objective:

To evaluate the cost-effectiveness of hospital-based (IVIg) and home-based (SCIg) treatment options for patients with primary immunodeficiency disorder (PID).

Method:

Data are collected from various sources, including surveys (health-related quality of life using the AQL-6D), administrative data (treatment costs) and literature (transition probabilities). We then developed a Markov cohort model to capture a typical patient's treatment pathway and used it to calculate the incremental cost, incremental outcome and the incremental cost effectiveness ratio. Sensitivity analyses were conducted to identify the influential factors, and to understand the effect of those factors on the cost-effectiveness result.

Results:

Results indicate that the home-based (SCIg) treatment is a cost-effective option for PID patients. Even though SCIg treatment occurs at shorter intervals (weekly), the medication cost is comparable to that of IVIg (monthly). Cost-savings from SCIg are achieved through reduced hospital outpatient cost. Home-based SCIg has the additional benefit of patients having less exposure to nosocomial infections and fewer hospital admissions, thus delaying the potential development of bronchiectasis.

Conclusion:

Home-based SCIg is an alternative treatment for patients with difficult venous access and for those who prefer the flexibility of home treatment. In addition, the model shows that SCIg treatment incurs lower costs to the health service provider.

391. Comparison of whole blood filter collection sets for cryopreservation of red cells

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Aim:

The aim of this study was to compare three different whole blood filter collection sets for their suitability to produce red cell concentrates (RCCs) acceptable for red cell cryopreservation.

Method:

Whole blood (470 mL) was collected into each of the collection sets; MacoPharma FQE6283LB, Fresenius PQT3949, and Haemonetics WBT434KCE (n=25 each). Whole blood collections were filtered at ambient temperature within 24 hours as per manufacturer's instructions. Whole blood was hard-centrifuged and separated into plasma and red cells using a semi-automated press. RCCs were sampled for quality testing then cryopreserved with 40% glycerol. Frozen red cells were thawed, deglycerolised using an ACP-215, resuspended in AS-3, and stored at 2-6 °C for 14 days. Units were sampled on the day of deglycerolisation and days 1, 7 and 14 post-deglycerolisation.

Results:

All MacoPharma, 89.5% of Fresenius and 95.8% of Haemonetics units met the leukoreduction specification ($<1.0 \times 10^6$ /unit). RCC volumes were 268 ± 17 , 278 ± 14 and 267 ± 17 mL for MacoPharma, Fresenius and Haemonetics packs respectively. The starting Hb of RCCs was 61 ± 6 , 63 ± 4 and 62 ± 3 g/unit for MacoPharma, Fresenius and Haemonetics packs, and whole blood Hb recovery post-deglycerolisation was 78, 75 and 75% respectively. Glucose was still detectable 14 days post-deglycerolisation whilst 2,3-DPG was below detection limits on day 7 post-deglycerolisation. Average haemolysis on day 14 post-deglycerolisation was 0.74 ± 0.20 , 0.41 ± 0.14 and $0.42 \pm 0.11\%$ for MacoPharma, Fresenius and Haemonetics packs respectively, with six MacoPharma units exceeding 0.8%, the upper limit of acceptance.

Conclusion:

All whole blood filter collection sets were found to be suitable for producing RCCs for cryopreservation. However, the Haemonetics whole blood filter pack was the only one to meet the Australian Red Cross Blood Service's specifications for leukocyte reduction and haemolysis in post-deglycerolisation red cell storage.

392. The effect of lipaemic plasma on red cell haemolysis

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Aim:

The aim of this study was to determine whether there is a correlation between the concentration of plasma lipoproteins and the extent of red cell haemolysis at expiry.

Method:

Forty five lipaemic whole blood donations were collected during a six week period. Red cell concentrates produced from these donations were stored at 2-6 °C, sampled on days 7, 21 and 42 of storage and tested for haemolysis and lactate dehydrogenase (LDH) release. Corresponding plasma were also obtained, rapidly frozen at -30 °C and batch tested. Plasma units were visually assessed for turbidity and categorised as mild (hazy), moderate (milky), high (creamy) and extremely high (creamy and viscous). Upon thawing concentrations of triglycerides, cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined.

Result:

Red cell haemolysis at expiry was $0.19 \pm 0.11\%$, which correlated with the LDH concentration 940 ± 678 U/L, $R^2=0.7584$; $p<0.0001$. Plasma triglyceride concentrations were 3.18 ± 1.95 mM, with 29 units falling in the normal range (0-300 mg/dL), 14 in the high range (300-600 mg/dL), and 3 very high (600-1000 mg/dL). By visual assessment, 14 plasma units were categorised as mildly lipaemic, 24 were moderate, 6 high and 2 extremely high, which did not correlate with the triglyceride categories. The mean cholesterol level was 5.45 ± 2.05 mM, with only eight units in the high range (>240 mg/dL). HDL concentrations were 0.65 ± 0.29 mM, whilst LDL/VLDL concentrations were 5.39 ± 1.27 mM. There was no correlation between haemolysis levels and triglyceride or cholesterol concentrations, $R^2<0.1$; $p=0.1458$ and 0.2141 respectively.

Conclusion:

There were no significant correlations between the level of lipoproteins in plasma and the percentage of red cell haemolysis at expiry. Red cells from lipaemic whole blood collections will continue to be routinely stored and issued.

393. ROTEM guided massive transfusion protocol: a regional hospital experience

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Aim:

Massive Transfusion Protocols (MTPs), if properly followed will improve patient outcome and reduce inappropriate blood product usage. This is even more important in regional/remote areas where access to blood products and specialist services are limited. Evidence suggests that use of ROTEM to guide massive transfusion will lead to reduction in blood product wastage without compromising outcome. We recently introduced a ROTEM guided MTP at Toowoomba Hospital, a regional hospital in Queensland. We report the results of an audit that was undertaken 9 months after introducing the ROTEM into our MTP to determine if blood product use was reduced.

Method:

Retrospective data was collected for patients presenting to Toowoomba Hospital Emergency Department who met criteria for Massive Transfusion 9 months before implementing a ROTEM guided MTP and 9 months after its introduction. The period of study was from January 2016 to 30 June 2017. The ROTEM MTP was introduced in October 2016. Inpatients were excluded from the study. Data on FBC, coagulation profiles and blood product usage were obtained from the hospital pathology system, Auslab.

Result:

With the installation of ROTEM, red cell and platelet usage was unchanged. There was significant reduction in Fresh Frozen Plasma usage. FFP wastage remained unchanged. Cryoprecipitate use was increased. There appeared to be no correlation between cryoprecipitate use and ROTEM results. Serial ROTEM was performed in only half the cases.

Conclusion:

There has been no significant change in red cell and platelet usage during MTPs despite introduction of the ROTEM. The FFP usage was significantly reduced but wastage was not. Cryoprecipitate usage was significantly increased. Further studies are required to determine the appropriate use of ROTEM during MTP and identify reasons why there has not been a reduction in blood product usage at our institution.

394. Comparing intravenous immunoglobulin associated thromboembolic events between autoimmune and immune deficient conditions

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Aim:

This pilot study aimed to estimate the local incidence of intravenous immunoglobulin (IVIG) associated thromboembolic events (TEE) and explore the hypothesis that those with autoimmune conditions have a higher incidence of IVIG associated TEE than conditions with hypogammaglobulinaemia, given a heightened concern among local neurologists compared to haematologists.

Method:

IVIG recipients in the Gold Coast area between 22/08/2016 and 01/03/2017 were identified from the Bloodstar database. Electronic medical records were reviewed to identify those who had ever suffered a TEE during, or in the 30 days following, administration of IVIG. The indication for IVIG and dose was collected. Duration of IVIG therapy was estimated based on commencement date and indication and incidence reported per 100 person-years. Assuming Poisson regression analysis the sample size required to show a doubling in incidence among those with autoimmune conditions compared to hypogammaglobulinaemia was calculated.

Results:

Five of 111 patients suffered an IVIG associated TEE with an incidence of 1.1 per 100 person-years of estimated IVIG use. Those with hypogammaglobulinaemia had an incidence of 1.2 per 100 person-years compared to 1.0 per 100 person-years for those with autoimmune conditions. However with only 251 and 199 person-years of estimated IVIG use in the hypogammaglobulinaemia and autoimmune arms respectively this study was underpowered. We calculated that 1973 person-years per arm would be needed to detect a doubling in incidence in the autoimmune arm.

Conclusion:

IVIG associated TEE is an uncommon complication. It remains unclear if a difference in incidence exists between different indications. Mandatory reporting of immunoglobulin associated complications, perhaps through the National Blood Authority haemovigilance program and Bloodstar may help generate the much larger sample sizes needed to study this and identify those at higher risk of TEE who may benefit from preventative measures.

395. Auditing ferric carboxymaltose use in a regional hospital for appropriate indication and dose adequacy

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Aim:

The aim was to assess whether patients in Toowoomba Hospital receiving ferric carboxymaltose (FCM) were being diagnosed with iron deficiency beforehand and whether the dose prescribed was adequate to replace the iron deficit calculated using the Ganzoni formula or Simplified Method.

Method:

All prescriptions for FCM dispensed at Toowoomba Hospital in 2015 were audited for baseline demographics, including presence of established chronic kidney disease (CKD) and pregnancy status, ferritin, transferrin saturation, dose of FCM prescribed, haemoglobin and weight. Iron deficit was calculated using the Ganzoni formula and Simplified method.

Results:

213 patients received 239 separate prescriptions for FCM. All 11 prescriptions (100%) for patients with CKD met the outlined definition for iron deficiency from a previous trial. 167 out of 228 prescriptions (73%) for patients without CKD met the definition for iron deficiency with iron studies not being performed in 60 out of 228 (26%). In patients with CKD the mean difference between the prescribed FCM dose and the iron deficit by Ganzoni formula and Simplified Method was 918mg and 955mg respectively. In patients without CKD the mean difference was 429mg by the Ganzoni formula and 545mg by the Simplified Method. Overall the iron deficit was fully replaced in only 15%.

Conclusion:

Clinicians are not consistently diagnosing iron deficiency before prescribing FCM, predominantly through not actually assessing iron status. This may result in FCM being prescribed inappropriately. Because of FCM's weekly dosing limit, patients are being under dosed which may result in unnecessary blood transfusions or repeat visits to hospital for additional iron replacement. Other intravenous iron preparations that allow a higher dose to be given on a single occasion should still be considered a viable alternative.

396. Review of platelet transfusion refractoriness referrals to the Australian Red Cross Blood Service in Queensland

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Background:

Platelet transfusion refractoriness (PTR) refers to an inadequate increase in platelet count after transfusion of a standard dose of platelets, commonly defined as a platelet increment of $<10 \times 10^9/L$ 1 hour post transfusion. Immune and, more commonly, non-immune factors contribute to PTR. Immune PTR is managed by transfusion with HLA/HPA compatible platelets provided by the Australian Red Cross Blood Service (Blood Service).

Aim:

To review the PTR referrals to the Blood Service (Queensland) over a two-year period to assess the clinical characteristics of the patient cohort and platelet products issued.

Method:

Cases between 1/8/2014 to 31/8/2016 were identified from the Blood Service special platelet support database. Retrospective review undertaken of clinical details, platelet counts and platelet product information.

Results:

79 referrals received, 68 unique patients, median age 60yo (range 10-85yo), 50 females (74%), history of pregnancy 26 (52%). Non-immune factors contributing in 43% cases.

Patient diagnoses include haematological malignancy 56 (82%), aplastic anaemia 6 (9%), solid organ malignancy 3 (4.5%); treated with chemotherapy 48 (71%), immunosuppression 6 (9%), nil 10 (14%), unknown 5 (6%).

60% referrals received HLA-compatible platelets. Median time from referral to commencing support 4 days (Interquartile range (IQR) 1-14), duration 29 days (IQR 10-107), median units of platelets issued 10 (IQR 4-21). Median best match (in house software) score 0 (fully matched) (IQR 0-0), worst 6 (IQR 2-8) (with < 8 acceptable).

Of 689 platelet units issued, 1hour increments provided for 24 transfusions (3%), ranging $0-59 \times 10^9/L$; 24hour increments provided for 270 transfusions (39%), ranging $0-90 \times 10^9/L$. Due to small numbers, further analysis not performed.

Conclusion:

Clinical characteristics of referred cases are in keeping with literature. Blood Service provides well matched platelet support however we are unable to accurately assess increment response. Ongoing engagement with clinicians will improve this transfusion support and the provision of follow-up data.

397. Prediction of cancer associated thrombosis

Pabinger I

Venous thromboembolism (VTE) is a frequent and burdensome complication in cancer patients, with an incidence that varies from 1-20% in different patient groups. Prophylactic anticoagulation approximately halves the relative risk of VTE in cancer patients – however, the absolute risk reduction of this intervention is modest for the majority of unselected ambulatory cancer patients, with a risk of 3-5% in the first months after diagnosis. Therefore appropriate tools are urgently needed to better discriminate between patients with high or low risk of VTE.

T

he most important risk factor for development of VTE in patients with cancer is the tumor site. There are high- and low-risk cancer sites. Patients with pancreatic, gastric or brain cancer are at the highest risk of VTE, patients with lung or colorectal cancer have an intermediate risk, whereas patients with breast and those with prostate carcinoma have a low risk.

To improve the risk prediction for the individual patient, scores have been developed. The first score described and validated was the Khorana score, including tumor site, blood count parameters and body mass index. There are other scores that include further variables, such as the Vienna risk score, which also includes biomarkers D-dimer and P-selectin. However, in a most recent validation these scores had only limited predictive value, just two out of four scores (the Vienna prediction score and the Protect score) allowed a notable discrimination between low- and high-risk patients.

An improvement in risk prediction is warranted to best categorize patients into high- and low-risk for decision on thrombosis prophylaxis in ambulatory cancer patients. Most recently we developed a score, which includes only two variables, the tumour site and D-Dimer plasma level. This score was externally and independently validated and showed good discrimination of patient with cancer at high or low risk of VTE.

398. Role of cancer screening in patients with thrombophlebitis and venous thromboembolism

Omani A

Abstract not supplied

399. Treatment of cancer associated thrombosis

Chen V

Concord General Hospital And Anzac Research Institute

Patients with cancer have a high risk of developing, and dying from, venous thromboembolism (VTE). Unique factors drive cancer associated thrombosis via an interaction between cancer biology and therapy related risk factors and current standard of care therapy is different for cancer versus non-cancer associated thrombosis. Randomised controlled trials and meta-analyses both demonstrate superiority of low-molecular-weight heparins (LMWH) over warfarin for recurrent VTE based on overall beneficial safety and efficacy. However, with patients living longer with metastatic cancer, LMWH heparin therapy can be associated with significant injection fatigue. Furthermore, there is still an increased rate of both bleeding and VTE recurrence in the cancer thrombosis population on LMWH. The direct oral anticoagulants (DOACs) rivaroxaban and apixaban, are approved for the treatment of acute VTE in Australia and are attractive alternative. Pooled sub-analyses of cancer patient within phase III trials, suggest that DOACs had equivalent efficacy and safety compared with VKA therapy for the treatment patients with cancer-associated VTE. However, the populations of cancer patients included in the DOAC trials compared with the dedicated LMWH trials for cancer associated thrombosis are not comparable with regard to mortality and VTE risk. Currently, there are no specific data from direct head-to-head comparisons of DOACs with LMWHs available for cancer associated thrombosis and use of DOACs for the management of VTE in cancer is not yet recommended by clinical practice guidelines. Results from direct comparison trials would greatly assist guide management in this difficult to manage population.

400. Neutrophil extracellular traps (NETS) and their dysregulation in multiorgan failure

Harrison P

Immuno-thrombosis forms a vital part of host protection against invading pathogens. This process is supported by innate immune cells and forms a matrix upon which recognition and elimination of pathogens occurs. Neutrophils also have a multifaceted role in coagulation and are now implicated in thrombosis, tissue damage and various disease pathologies. One proposed mechanism by which this occurs is through the generation of neutrophil extracellular traps (NETs). NETosis is a tightly regulated cell death pathway that results in the extrusion of chromatin to the exterior of the cell. NETs have been shown both in vitro and in vivo to ensnare, trap and in some instances directly eliminate pathogens. Recent studies have shown that NETs can initiate a pro-coagulant phenotype and mediate both tissue and organ damage. NET induced thrombi have decreased permeability, decreased susceptibility to fibrinolysis and increased clot stability. NETs are also capable of perturbing blood flow through capillary networks which may result in tissue hypoxia and the promotion of disseminated intravascular coagulation and the pathogenesis of multi-organ failure (MOF). We measured peripheral blood neutrophil function and biomarkers of NETosis in a cohort of severely burn-injured patients who were monitored for the development of sepsis/MOF and underwent serial sampling over one year post-injury. We found that neutrophil dysfunction and ex-vivo NET formation was also significantly compromised in septic patients, suggesting that this may predispose patients to bacterial infection. Despite this plasma cfDNA levels following thermal injury were significantly increased in those patients who developed sepsis but were also cleared effectively upon recovery. High levels of Citrullinated Histone H3 also coincided with the maximal levels of cfDNA, demonstrating that NETosis is occurring. PCR analysis of extracted DNA from plasma samples confirmed that the majority of the cfDNA was of nuclear origin and was elevated in MOF. DNase activity (a major regulator of NETs) was also reduced up to a month post-injury and was significantly lower in patients with MOF. Actin (a direct inhibitor of DNase) was detectable after injury & during sepsis/MOF. The actin scavenger proteins Gelsolin (GSN) and Vitamin D binding protein (VDBP) were also significantly depleted post-injury. To investigate the potential therapeutic use of GSN/VDBP we performed a preliminary retrospective analysis in samples from military patients receiving fresh frozen plasma (FFP) prior to first blood sampling and hospital admission following severe poly-trauma sustained in explosions. Pre-hospital administration of FFP significantly increased both GSN and DNase levels immediately after severe poly-trauma. Restoring the actin scavenging system therefore has clinical potential. We conclude that severe injuries induce release of actin that depletes the actin scavenging system resulting in direct inhibition of DNase and pre-disposition to uncontrolled NETosis during sepsis/MOF.

401. Essential role of ERp5 in thrombosis

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The oxidoreductase, endoplasmic reticulum protein 5 (ERp5), is released at the site of thrombus formation and ERp5 inhibitors reduce platelet accumulation, fibrin generation and thrombus formation in mice. ERp5 is secreted by platelets and endothelial cells and binds to surface $\beta 3$ integrin receptor. The ERp5 binding site has been localised to the βI domain of the $\beta 3$ subunit of the major platelet integrin, $\alpha 2\beta 3$. The oxidoreductase activity of ERp5 suggested that the enzyme cleaves one or more disulphide bonds in the integrin. We have measured the redox state of 31 of the 37 disulphide bonds in $\alpha 2\beta 3$ integrin using differential cysteine alkylation and mass spectrometry. Incubation of RGD-extended $\alpha 2\beta 3$ integrin with ERp5 resulted in significant cleavage of only one of the 31 disulphide bonds – the Cys177-Cys184 disulphide bond in the βI domain where ERp5 binds. The Cys177-Cys184 bond lies at the rim of the fibrinogen binding pocket of the integrin and molecular dynamics studies reveal that cleavage of the disulphide changes the ligand binding pocket and the nature of the fibrinogen interaction. This has been confirmed in single molecule force spectroscopy experiments of ERp5 effect on $\alpha 2\beta 3$ integrin binding to fibrinogen, and flow studies of ERp5 effect on platelet binding to immobilised fibrinogen. Based on these findings, we can conclude that ERp5 released during thrombus formation negatively regulates fibrinogen binding to $\alpha 2\beta 3$ integrin by allosterically modulating the binding site. We suggest that ERp5 mediates fibrinogen release from activated integrin that enables dynamic platelet adhesion and spreading.

402. Thiol isomerases and their role in thrombosis

Furie B

Harvard Medical School

Abstract not provided

403. Fibrin-mediated GPVI shedding in patients with trauma or inflammation

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Aim:

Ligand engagement of platelet glycoprotein VI (GPVI) by collagen activates α IIb β 3 and Metalloproteinase 10 (ADAM10)-mediated release of soluble GPVI (sGPVI). Fibrin also binds GPVI. We assessed whether fibrin-GPVI interaction triggered shedding in vitro and establish if fibrin-induced shedding mediates sGPVI elevations in inflammatory and injured patients

Methods:

Washed platelets were exposed to experimental conditions that induce shedding of GPVI (FX activation by Russell viper venom, shear stress) or fibrin by treatment with thrombin (1U/mL) in the presence of fibrinogen (100 μ g/mL). Intact and cleaved GPVI were quantitated by western blot, densitometry and ELISA in the presence/absence of metalloproteinase inhibitors, GPRP peptide, to assess fibrin polymerization, or rivaroxaban (1 μ g/mL; direct Factor X (FX) inhibitor). Plasma sGPVI levels and D-dimer levels (a fibrin-degradation product) were measured in temporal samples from thermal injury (n=99) or ICU patients (n=83).

Results:

sGPVI levels associated with 28-day mortality in ICU patients (p=0.02). sGPVI positively correlated with D-dimer levels at day of ICU admission (r=0.41) and at day-14 in thermal injury patients (r=0.46). Fibrin induced metalloproteolytic shedding, reducing intact GPVI levels by 67%. GPCR agonists (ADP, PAR1 and PAR4-activating peptides and thromboxane) did not induce shedding. Fibrin-mediated GPVI shedding was independent of integrin α IIb β 3 and FXa but required fibrin polymerization and metalloproteinase activity. Specific ADAM10 activity and GPVI signalling through Src/Syk, did not have major roles in fibrin-mediated shedding. Together the data suggests roles for multiple sheddases. Shear stress in conjunction with fibrin exposure reduced intact GPVI levels, as measured by flow cytometry.

Conclusions:

Fibrin induces shedding of GPVI in vitro, providing a plausible mechanism of elevation of sGPVI in acute injury patients. Elevated sGPVI was predictive of mortality, where minimal collagen exposure is assumed. Fibrin degradation strongly correlated with sGPVI in both patient cohorts. Fibrin-induced GPVI shedding may contribute to sGPVI elevation in these patients.

404. The role of platelets in increased thrombogenicity following splenectomy: Key platelet-specific receptors and activation markers

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Aim:

The mechanisms underlying increased thrombogenicity in splenectomy are poorly understood. We assessed a potential role for platelets by analysing surface expression of key platelet-specific receptors glycoprotein(GP)Ib α , GPVI and α IIb β 3, and a platelet activation marker, P-selectin. GPIb α , that binds von Willebrand factor and other ligands, is constitutively shed from aging platelets, and its surface density is crucial for both thrombus formation and platelet clearance through interactions with phagocytic cells. GPIb α also forms a non-covalent complex with GPVI, which binds collagen and fibrin. GPIb α /GPVI initiate platelet adhesion, leading to platelet activation and aggregation, secretion of procoagulant factors and thrombus formation.

Methods:

Whole blood from same-day healthy controls ($n=9$) or splenectomy ($n=18$) was centrifuged to obtain platelet-rich-plasma, and stained with phycoerythrin(PE)-labelled antibodies against GPIb α (PE-AK2), GPVI (PE-1G5), α IIb β 3 (PE-CD41a), CD9 (PE-CD9), and P-selectin (PE-CD62P) using standardized protocols, and analysed using a FACSCalibur.

Results:

Preliminary analysis suggested that compared to healthy controls, platelets from splenectomy cases showed significantly decreased surface expression of GPIb α ($p<0.0001$) and GPVI ($p=0.0009$), as well as CD9 ($p=0.0069$). In contrast, there was no significant difference in relative surface expression of α IIb β 3 ($p=0.718$). Further, consistent with increased platelet activation/secretion, expression of surface P-selectin was more variable and significantly elevated in splenectomy *cf.* control platelets ($p=0.0132$). ELISA analysis of stored plasma will be used to confirm whether decreased platelet GPVI is likely due to increased shedding, resulting in increased plasma sGPVI.

Conclusion:

The results of this pilot study support a role for hyperactivated platelets post-splenectomy, and identify platelet-specific markers related to these changes. Methods and results developed here will enable future larger temporal studies of platelet-related factors, and determine if such measurable changes correlate with clinical haemostatic/thrombotic dysfunction. This can also help explain basic mechanism(s) of thrombotic propensity in this population, and/or indicate potential therapeutic targets for prevention of thrombosis.

405. The role of platelet-attached glycans in platelet function

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Background:

The platelet membrane contains many highly branched carbohydrate chains; which are capped by sialic acid. These glycans can be cleaved off the surface and this has been implicated in the clearance of senescent and cold-stored platelets, as well as in immune thrombocytopaenia patients. The majority of glycans are attached to one of the main platelet adhesion receptors, glycoprotein (GP)Ib α . So far, four different forms of the enzyme responsible for cleaving sialic acid have been identified in mammals (neuraminidases, NEU1-4); however their role in platelet function is largely unknown.

Aim:

To study the potential role of glycans and neuraminidases in platelets.

Method:

Donors were consented to donate either whole-blood (to obtain PRP) or apheresis platelets (n=8). Platelet rich plasma (PRP) was stimulated with ristocetin, ADP and arachidonic acid (n=6). NEU1 and NEU2 membrane expression was measured by flow cytometry, as were platelet-attached glycans using *Ricinus Communis Agglutinin-1* (RCA-1; detecting galactose) and Wheat Germ Agglutinin (WGA; detecting sialic acid and N-acetyl-D-glucosamine, GlcNAc). GPIIb/IIIa-integrin and/or GPIb α mediated signalling was inhibited by RGDS, addition of GlcNAc or O-sialo-glyco-endopeptidase cleavage respectively. Apheresis platelets were studied on day 1, 2, 5, 7, 9 post-collection.

Result:

Activation of GPIb α by ristocetin induced a 3-fold increase in RCA-1 binding (p<0.05), and reduced WGA binding (p<0.05), while stimulation by ADP or AA showed no effect. Interestingly, basal membrane expression of both NEU1 and 2 was found, which increased by 5- and 3-fold respectively following ristocetin stimulation (p<0.05). Inhibition of GPIIb/IIIa-integrin inhibited NEU1 expression. More importantly, GPIb α inhibition and/or cleavage of its extracellular part decreased the majority of membrane-associated NEU1 and NEU2. In apheresis platelets, ristocetin stimulation increased cleavage of sialic acid significantly, and was found to be highly variable between donors.

Conclusion:

These results show a potential novel role for NEU1 and NEU2 in platelet activation, which is highly dependent on GPIb α -mediated signalling.

406. Age-related changes in platelet activation pathways

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Aims: Cardiovascular disease is a leading cause of mortality in the elderly. However, platelet activation, which is pivotal to atherothrombosis, is poorly understood in healthy older populations. We investigated whether there is a higher basal platelet activity and/or differential agonist-mediated upregulation of platelet activation pathways in the elderly.

Methods: We performed a prospective observational study recruiting patients and staff at Concord Hospital in the following age groups: (i) 20-30 (young); (ii) 40-55 (middle age); (iii) ≥70 years (elderly). Platelet activity was assessed by whole blood aggregometry (Multiplate[®]) and platelet surface markers (CD62P alpha granule release, CD63 dense granule release, and the GPIIb/IIIa conformational activation marker PAC-1) measured by flow cytometry under basal conditions and after agonist stimulation (adenosine diphosphate [ADP] 0.25-1.25uM, thrombin 0.05-0.5IU/ml).

Results: Circulating platelets demonstrated increased basal platelet expression of CD63, PAC1 and CD62P in older subjects compared to young subjects (Figure 1, n=16), and increased PAC-1 binding and CD63 expression in older subjects post ADP stimulation with higher maximal response and lower EC50. However, older populations had lower PAC-1 binding and CD63 expression post thrombin stimulation than did middle aged and younger populations. There was no difference between middle-aged and older individuals in CD62P expression. Ageing was associated with an increasing platelet aggregation response to ADP 6.67uM (n=21, ANOVA p=0.0016, linear trend for three groups p=0.009).

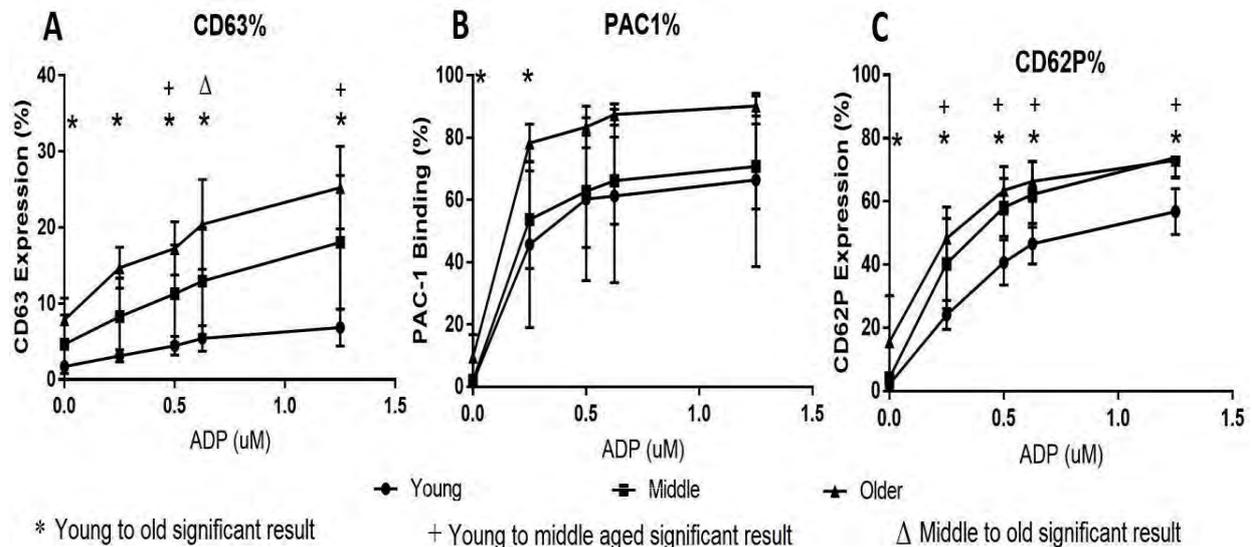


Figure 1: Platelet activation post ADP stimulation (mean±SD, n=6 young, 5 middle-aged and 5 elderly subjects): (A) CD63 expression; (B) PAC-1 binding; (C) CD62P expression.

Conclusion: These preliminary results indicate that platelet activation pathways are differentially altered with ageing. Understanding age-related changes may pave the way for novel and age-specific antiplatelet strategies.

407. 2'OMe oligonucleotides block the miR-494/PROS1 interaction in patients with PS deficiencies

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Aim:

Protein S (PS) is expressed by the gene *PROS1* which is subject to negative regulation via miR-494 binding to 3 sites found within the 3'UTR. Patients with a hereditary *PROS1* mutation have PS deficiencies, that can be exacerbated by miR-494 regulation, leading to possible deep vein thrombosis, pulmonary embolism, or other potentially life-threatening thrombotic complications. This study aimed to evaluate specifically targeted 2' O-methyl (2'OMe) phosphorothioate modified oligonucleotides designed to block the miR-494/*PROS1* interactions, increasing the *PROS1* mRNA transcript lifespan leading to an overall increased expression of PS.

Method:

HuH-7 cells were transfected with either pre-miR-494 or a scrambled control pre-miR-NC with and without 2'OMe oligonucleotide blockers specific to each of the 3 sites. The direct interaction between the miR-494, oligonucleotide blockers and the *PROS1* 3'UTR was determined using a dual-luciferase reporter vector transient transfection system.

Results:

The dual-luciferase reporter assay confirmed the downregulation of *PROS1* mRNA through miR-494 binding by ~40%. The introduction of 2'OMe blockers at sites 2 and 3 along with miR-494 abrogated this response, when compared to the negative control.

Conclusion:

The addition of 2'OMe blockers leads to the successful blocking of the miR-494/*PROS1* interaction, particularly at sites 2 and 3. Preliminary results support the aim, showing an increase in *PROS1* 3'UTR dependent expression, with subsequent western blotting and qPCR to follow to confirm increases in *PROS1* mRNA and PS antigen. 2'OMe oligonucleotides are stable in plasma, however further evaluation and possible modification for nuclease resistance is needed if they are to be considered for use as an *in vivo* agent for patients with hereditary PS deficiencies.

Conflict of Interest:

No conflict of interest to disclose

408. A common protease-activated receptor 4 variant causes platelet hyper-reactivity and its blockade is antithrombotic

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Background:

Thrombin activates platelets via two protease-activated receptors (PARs), PAR1 and PAR4, and targeting PARs is an emerging anti-thrombotic approach. We have previously shown that selective inhibition of PAR4, but not of PAR1, impairs thrombin generation and fibrin formation in the setting of thrombosis, thereby rationalising PAR4 antagonism as a novel anti-platelet strategy. However, a recent landmark study described a commonly expressed PAR4 variant (rs773902; encoding either Ala120 or Thr120), where the presence of the Thr120 allele can be found in up to 80% of people depending on geographical location. Here, we report that this variant is associated with a procoagulant platelet phenotype, and inhibition provides significant anti-thrombotic effects.

Aim:

To examine PAR4 activation and inhibition of Ala120 and Thr120 PAR4 variants.

Methods:

PAR4 activation and inhibition were examined in: 1) cultured cells transfected with human PAR4 variants; 2) human isolated platelets and; 3) human whole blood thrombosis.

Results:

We found that platelets from donors with the Thr120 variant have increased sensitivity to thrombin-induced platelet aggregation and phosphatidylserine exposure, indicating these individuals have a heightened procoagulant-platelet phenotype that is PAR4-dependent. To examine inhibition of the receptor, we transfected HEK293T cells with Ala120 or Thr120 PAR4 variants, and found PAR4 inhibition provided near-complete blockade of thrombin activation regardless of variant status. Further, we were able to inhibit PAR4-dependent platelet aggregation and phosphatidylserine exposure in both Ala120 and Thr120 donors. Finally, in the setting of whole blood thrombosis, we found that PAR4 inhibition abolished thrombin generation (FRET-based thrombin substrate) and fibrin formation (anti-fibrin antibody) across all PAR4 genotypes.

Conclusion:

These findings indicate that the Thr120 PAR4 variant is associated with a hyper-responsive platelet phenotype which is able to be inhibited by selectively blocking PAR4, thus providing further rationale for PAR4 antagonism as a novel anti-thrombotic approach.

409. Platelets in regenerative medicine

Harrison P

Platelets are increasingly recognised to play important roles in physiology beyond promotion of haemostasis and thrombosis. Platelet-Rich Plasma (PRP), an autologous derivative of whole blood that contains a supra-physiological concentration of platelets, has gained increasing attention in both the scientific literature and the wider media (e.g. with high profile sports personalities) for its potential application as a regenerative adjunct therapy. The regenerative effect of PRP exerted by producing a local environment for tissue regeneration which is rich in growth factors and other cytokines has been supported by in vitro and animal studies that suggest a positive influence on the migration and proliferation of a number of cell types. Furthermore, the full array of potential bioactive growth factors and chemokines released upon platelet activation is becoming well defined. These include transforming growth factor (TGF- α 1 and α 2), platelet-derived growth factor (PDGF-AA, AB and BB), vascular endothelial growth factor (VEGF A and C), Insulin growth factor (IGF-1) and epidermal growth factor (EGF). These factors can promote local angiogenesis, stem cell homing, local cell migration, proliferation and differentiation coupled with the deposition of matrix proteins such as collagen which all play a key role in enabling the restoration of normal tissue structure and function. The reported clinical use of PRP is largely confined to the last two decades and initially centred on its application in dental and maxillofacial surgery. More recently, regenerative effects of PRP in a range of tissue types including bone, cartilage, tendon and muscle have attracted interest in fields such as orthopaedic and plastic surgery where effective restoration of sometimes poorly vascularised and damaged tissue is a critical determinant of successful clinical outcome. Given the rudimentary knowledge of the mechanism of action of PRP in clinical settings and the limitations of current studies, it is important that any future clinical trial should be carefully designed not only with adequate power to accurately determine the clinical effect of PRP but also use disease-specific outcome tools. This has been highlighted by several authors, who have shown that the imprecision in effect size estimates from underpowered studies into PRP have led to unsafe conclusions. Also the field is not only plagued with poor standardization in the methods used to generate PRP but great variability in the purity and quality of products obtained and given to patients. The ISTH platelet physiology SSC has recently defined new guidelines for the use of platelets in regenerative medicine by performing a RAND survey conducted by a panel of experts. This includes 1) RCT design 2) Clear inclusion/exclusion criteria 3) Homogenous study population or stratification of variables 4) Standardised clinical assessment 5) Validated PRP production methods and delivery 6) Definition of PRP content (e.g. purity, cell counts and quality) 7) Robust outcome measures and 8) Standardised post treatment follow-up protocols. Currently, there is only one ongoing randomised clinical trial that includes all the above and is adequately powered to measure the true efficacy of PRP in clinical settings. The PATH-2 (Platelets in Achilles tendon Healing) trial in the UK will shortly finish recruiting patients (N = 214) and is now due to finish in 2018 after clinical follow up. Meanwhile, autologous PRP administration remains an attractive and popular strategy in many clinical scenarios given its cost-effective, minimally invasive and safe nature of the therapy. Large well designed clinical trials in the future will determine whether PRP is truly effective or not.

410. The proinflammatory function of dying platelets

Jackson S

Gut ischemia is common in critically ill patients, promoting thrombosis and inflammation in distant organs. The mechanisms linking hemodynamic changes in the gut to remote organ thrombosis remain ill-defined. Here we demonstrate that gut ischemia in the mouse induces a distinct pulmonary thrombotic disorder triggered by neutrophil macro-aggregates. These neutrophil aggregates lead to widespread occlusion of pulmonary arteries, veins and the microvasculature. A similar pulmonary neutrophil-rich thrombotic response occurred in humans with the acute respiratory distress syndrome. Intravital microscopy during gut I/R injury revealed rolling neutrophils extract large membrane fragments from remnant dying platelets in multiple organs. These platelet fragments bridge adjacent neutrophils to facilitate macro-aggregation. Specific-deletion of cyclophilin D from platelets prevented neutrophil macro-aggregation and pulmonary thrombosis. Our studies demonstrate the existence of a distinct pulmonary thrombotic disorder triggered by dying platelets and neutrophil macro-aggregates. Therapeutic targeting of platelet death pathways may reduce pulmonary thrombosis in critically ill patients.

411. Platelet receptor levels in health and disease

Gardiner E

The two platelet receptors that initiate platelet activation are the glycoprotein (GP) Ib-IX-V complex and the GPVI/FcR α -chain. These receptors are co-associated and form a unique adhesion-signalling complex. Co-operatively, they initiate and propagate both haemostasis and thrombosis. In the arterial circulation when an atherosclerotic plaque ruptures, GPIb-IX-V and GPVI/FcR α instantly detect disturbances in blood flow (changes to fluid shear stress) and initiate adhesion to exposed thrombogenic materials.

A consequence of exposure of human platelets to thrombogenic collagen or fibrin is that platelets shed the adhesion/signalling receptor GPVI. This process is mediated by ADAMs (A Disintegrin And Metalloproteinase) and is a signature activity of this family of membrane surface metalloproteinases. The ligand binding portion of GPIb-IX-V (GPIb α) is also metalloproteolytically shed. Platelet GPVI and GPIb α (i) respond to changes in vessel shear stress which can result in thrombus formation, for example in partly-occluded coronary vessels; (ii) receptor shedding occurring during and after exposure to shear is relevant to thrombus propagation and stability e.g. infarction or embolization are events controlled by receptor levels/shedding; (iii) shear-dependent receptor shedding clearly occurs in human plasma, underscoring the pathological relevance of these events.

We have developed confocal microscopy approaches to simultaneously quantitate receptor levels, ADAMs activity, areas of thrombus growth or loss and a range of fluid rheological parameters (shear, shear gradients, velocity, pressure variations, pulsatility) on the surface and inside a forming thrombus. Using our standardized platelet flow cytometry assays developed in-house, we have demonstrated that there is significant reduction in platelet GPIb α and GPVI surface levels, and concomitant increases in sGPVI in patients exposed to altered or elevated blood fluid shear stress such as patients with aortic stenosis, or in patients receiving mechanical circulatory support through extracorporeal membrane oxygenation (ECMO) or left ventricular assist devices (LVADs) compared to the healthy donors. Platelet receptor levels and levels of receptor fragments in plasma may aid in the evaluation of patient thrombotic potential and bleeding risk.

412. miR-494-3p regulates transcription factors Sp1 and STAT5B to modulate haemostatic factor expression

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Aim:

Oestrogen can modulate Protein S (*PROS1*) expression via miR-494-3p in in vitro models. Several biological functions have been ascribed to miR-494 and we had previously postulated a role for it in oestrogen-mediated acquired Protein S deficiency. This study aims to: 1. Characterise the effects of miR-494 on the expression of coagulation factors at the mRNA and protein level. 2. Determine if miR-494 indirectly regulates coagulation factors by directly inhibiting transcriptional factors AP1, Sp1 and/or STAT5B.

Method:

HuH-7 human hepatoma cells were transfected with synthetic negative control miRNA (miR-NC) or miR-494, and the levels of mRNA and protein were determined with qPCR and Western blot, respectively, for; PS (*PROS1*); Plasminogen (*PLG*); and Tissue Factor (*F3*) at 48h post transfection. In silico analyses of 3'UTR sequences of transcription factors AP1 (*JUN*), Sp1 (*SP1*) and STAT5B (*STAT5B*) were performed to identify putative miR-494 binding sites. Computational transcription factor binding analyses (PROMO) was also performed on *PLG* and *F3* promoter regions.

Results:

Treatment of HuH-7 cells with miR-494 significantly downregulated *PROS1*, *PLG*, *SP1* and *STAT5B* mRNA and protein levels, with no changes observed for *JUN*. In contrast, *F3* mRNA expression was significantly increased with miR-494 treatment, but levels of tissue factor protein remained relatively unchanged. *JUN*, *SP1* and *STAT5B* 3'UTRs contained putative miR-494 binding sites, and PROMO analyses identified predicted AP1 binding sites in *PLG* and *F3* promoters, and Sp1 binding sites in the *F3* promoter. No STAT5B binding sites were identified.

Conclusion

Coagulation regulation by miR-494 is mediated via direct and indirect targeting of multiple coagulation factors. Direct targets such as *PROS1*, *SP1* and *STAT5B*, results in indirect regulation of *PLG* and *F3*, demonstrating a prothrombotic role for miR-494 and strongly suggests important clinical implications of raised miR-494 levels under high circulating oestrogen levels such as pregnancy.

413. A unique regional outreach program to provide targeted quality care to individuals with bleeding disorders

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Aim:

Timely access to appropriate medical care is a well-recognised challenge for patients with bleeding disorders in rural areas. Designated haemophilia treatment centres (HTC) aim to provide comprehensive care for these individuals. However emergency management at critical time periods for these patients is challenging due to the distance from the HTC.

Method:

We orchestrated a novel regional outreach program involving the key local stakeholders with the aim to provide quality care to individuals with haemophilia in rural NSW areas and greater ACT. The project goals were to provide a local platform of quality care through education and appropriate support offered to local health professionals, routine supply of clotting factor concentrates and enhanced bilateral communication between the HTC and local care providers. The pilot places selected for this program included Boorowa, Moruya and Albury due to the high demand for these services. We analysed the demographics of the participants of the education program, improvement in knowledge post education by a questionnaire and impact of the outreach program.

Results:

The three pilot sites have completed final stages now. Majority (31.8%) of the attendees were health professionals followed by individuals with bleeding disorders (18.1%). Approximately half of the audience completed the questionnaire pre and post education session. There was considerable improvement in knowledge post education (average increase in right answers by 48.6%). GP's, hospital staff and the ambulance service are equipped to provide factor administration locally since the introduction of the program. There are at least 18 recorded instances of factor administration in Boorowa hospital.

Conclusion:

Haemophilia patients can now get timely treatment at local hospitals due to increased awareness and availability of clotting factor supply. This quality improvement project highlights the importance of education and involvement of local health professionals in improving the care of individuals with bleeding disorders.

414. Utility of global coagulation assays in myeloproliferative neoplasm: thromboelastography, thrombin generation and overall haemostatic potential

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Aim: Myeloproliferative neoplasm (MPN), such as polycythaemia vera (PV) and essential thrombocythaemia (ET), are independent risk factors for cardiovascular and thrombotic events. However, there are no routinely available laboratory tests to evaluate the patient's thrombotic risk beyond the assessment of blood counts. Global coagulation assays such as thromboelastography, thrombin and fibrin generation may be better surrogate measures of thrombosis.

Method: Participants with MPN (proven on bone marrow biopsy and/or molecular diagnosis) were recruited. Thromboelastography (TEG® 5000) was performed on citrated whole blood while thrombin generation using calibrated automated thrombogram (CAT) and fibrin generation using overall haemostatic potential (OHP) assays were done on stored platelet-poor plasma.

Result: Thirty-eight MPN patients (20 females, 18 males) with median age 65 years (range: 29-84) were recruited. There were 26 patients with ET (68.4%), 8 PV (20.5%), 3 primary myelofibrosis and one with MPN, unclassifiable. When compared to normal controls, there was no significant difference in maximum amplitude (clot strength) although the lysis time (LY30) was significantly higher (5.1% vs 0.9%, $p < 0.01$) using thromboelastography independent of the use of aspirin. CAT parameters, however, showed higher thrombin peak 260.8 vs 227.3 nM; $p = 0.01$) and velocity index (93.4 vs 70.1 nM.min; $p < 0.01$) but preserved endogenous thrombin potential. Higher LY30 (>5%) was associated with higher haemoglobin (150.3 vs 138.4 g/L, $p = 0.03$) but not platelet count. Fibrin generation parameters (overall coagulation potential (OCP) and OHP) were significantly reduced in the MPN cohort ($p < 0.01$) with preserved overall fibrinolytic potential (OFP).

Conclusion: This study demonstrates a strong association between MPN with high lysis time and a reduction in fibrin generation parameters, which is contradictory to the prothrombotic nature of MPN. It may be a protective prognostic marker and could represent an underlying compensatory effect. Further prospective clinical studies to evaluate and confirm these findings are proposed.

	MPN (n=38)	Normal controls (n=41)	p-value
Haemoglobin (g/dL)	140.5	142.0	0.73
White cell ($\times 10^9/L$)	6.6	5.7	0.04
Platelet ($\times 10^9/L$)	526.0	237.5	<0.01
TEG Parameters			
R time (min)	7.2 \pm 2.4	6.4 \pm 1.9	0.14
Maximum amplitude (mm)	59.6 \pm 8.7	60.1 \pm 5.5	0.42
LY30 (%)	5.1 \pm 6.9	0.9 \pm 1.3	0.001
CAT Parameters			
Endogenous thrombin potential (nM.min)	1397.5 \pm 264.0	1363.0 \pm 216.0	0.52
Peak thrombin (nM)	260.8 \pm 54.9	227.3 \pm 61.1	0.01
Velocity index (nM.min)	93.4 \pm 31.8	70.1 \pm 29.8	0.001
OHP Parameters			
OCP	56.5 \pm 10.4	66.7 \pm 8.6	<0.001
OHP	27.4 \pm 5.1	33.0 \pm 7.0	<0.001
OFP (%)	51.3 \pm 5.1	50.6 \pm 8.3	0.66

415. Dental Extractions on NOACs without Stopping Therapy (DENTST) study: interim analysis

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Background/Aim: International guidelines recommend warfarin be continued for dental extractions. Our aim is to determine the safety of performing dental extractions on patients taking NOACs. Secondary aims are:

- to identify factors associated with increased bleeding risk
- 1. to determine if there is a safe lower limit of NOAC drug level below which dental extractions may be safely performed.

Method: This is a prospective cohort study with 3 groups: patients on NOAC, warfarin, or no anticoagulant. Participants do not withhold their anticoagulant. Blood tests are measured immediately prior to extraction. After extraction Surgicel is placed in the socket, the socket is sutured then pressure applied with gauze. The gauze is weighed before and after haemostasis is achieved. Bleeding complications are assessed at 48 hours and 7 days.

Results: Recruitment commenced in February 2016 with a target completion of November 2018. Data from 53 participants available at the time of abstract submission is summarised in table 1. Most patients on a NOAC had therapeutic dosage range drug levels. There has been no significant difference in bleeding rates between patients on warfarin and NOACs (4 patients [44%] and 12 patients [39%] respectively with any bleeding, P=1.0). Clinically relevant non-major bleeds (CRNMB) have only occurred in patients on warfarin, none on NOACs (2 patients [22%] and 0 patients respectively, P=0.046). There has been no significant difference in bleeding rates between the NOACs (4 patients [31%] on apixaban, 2 patients [33%] on dabigatran, 6 patients [50%] on rivaroxaban, P=0.635). The weight difference of gauze applied to the socket between the groups is small and clinically insignificant.

Table 1. Patient details and outcomes

Oral anticoagulant	No anticoagulant	Warfarin	All NOACs	Apixaban	Dabigatran	Rivaroxaban
Patient number	13	9	31	13	6	12
Total number of teeth extracted	27	19	62	18	18	26
Total number of roots extracted	48	30	83	28	22	33
Any bleeding – no. (%)	1 (8)	4 (44)	12 (39)	4 (31)	2 (33)	6 (50)
Major bleeding episodes	0	0	0	0	0	0
CRNMB episodes – no. (%)	0	2 (22)	0	0	0	0
Minor bleeding episodes - no.(%)	1 (8)	2 (22)	12 (39)	4 (31)	2 (33)	6 (50)
Mean (±SD) weight difference of gauze per tooth (grams)	2.5±1.9	2.5±1.7	2.6±2.2	3.1±2.8	1.7±1.5	2.5±1.5
Mean (±SD) weight difference of gauze per root (grams)	1.2±0.6	1.7±1.5	2.0±1.6	2.3±1.9	1.5±1.4	2.0±1.3
Assay	-	INR	-	Anti-Xa	Dilute thrombin time	Anti-Xa
Mean drug level (range)	-	2.2 (2.0-2.6)	-	130.4 ng/ml (37.3-238.7)	112.6 ng/ml (68.5-188.1)	137.7 ng/ml (11.0-487.0)

Conclusion: Interim results suggest that bleeding outcomes in patients on NOACs are comparable to patients on warfarin. NOAC interruption may be unnecessary for dental extractions.

416. D-dimer and residual thrombus can stratify risk of recurrent venous thromboembolic events

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Aim: Abnormal D-dimer after cessation of anticoagulation is a well-recognised risk factor for recurrent venous thromboembolic events (VTE). In Australia however, D-dimer has not been widely used to risk stratify VTE recurrence. Our aim in this study was to retrospectively analyse the relationship between post-treatment D-dimer, residual thrombus and VTE recurrence.

Methods: Medical records of patients attending a tertiary hospital with a diagnosis of VTE between January 2013 and June 2015 were retrospectively reviewed. The median follow up was 10 months (0 – 50 months).

Results: The total number of reviewed patients was 443. Of these, 101 patients (41 males, 60 females) had post-treatment D-dimer testing. Patients with abnormal D-dimer (>500ng/mL) had a higher rate of recurrence (31% vs 6.1%, ORR 7.1, 95%CI 2.1-24.5; p=0.0019). Eleven patients had anticoagulation recommenced as a result of elevated D-dimer – none of these patients experienced recurrent VTE. Table 1 displays rates of VTE recurrence stratified by D-dimer.

Table 1

D-dimer (ng/mL)	Pts with recurrent VTE	Pts without anticoagulation recommenced	Recurrence rate	Relative risk (compared to patients with normal D-dimer)
>1000	4	4	100%	16.3 (95%CI 6.3-42; p<0.0001)
500-1000	7	21	33.3%	5.4 (95%CI 1.8-16.7; p=0.0033)
<500	4	65	6.2%	

In patients with D-dimer between 500-1000ng/mL, age-adjustment was performed by using the patient's age x10. Recurrence rate in those with normal age-adjusted D-dimer was 20% (2 of 10 patients); in abnormal age-adjusted D-dimer this was 45.5% (5 of 11 patients). Table 2 displays stratification of patients using a combination of D-dimer and residual thrombus. Patients with normal D-dimer and no residual thrombus had the lowest rate of recurrence, with a trend towards significance - RR 0.15 (95%CI 0.02-1.34; p=0.09)

Table 2

D-dimer = DD	No. patients	Patients with recurrent VTE	Recurrence rate
Normal DD & residual thrombus	20	3	15%
Normal DD & NO residual thrombus	45	1	2.2%
High DD & residual thrombus	15	4	26.7%
High DD & no residual thrombus	18	6	33.3%

*3 patients did not have repeat imaging.

Conclusion: Post-treatment D-dimer can stratify the risk of VTE recurrence, with the highest in those with D-dimer >1000ng/ml. D-dimer between 500-1000ng/mL conveys an intermediate risk of recurrence; in these patients age adjustment may further stratify risk. Presence of residual thrombus has relevance in patients with normal post-treatment D-dimer, and may be associated with a higher risk of recurrence. This information can help to identify patients suitable for long-term prophylaxis, now a feasible and safe option in the era of novel anticoagulants.

417. Use of thromboelastography to identify patients with acquired coagulopathy at risk of developing thromboembolic events.

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Aims:

Recent evidence suggests that patients with abnormal coagulation profiles remain at risk of developing thromboembolic events. This prospective cohort study aimed to assess the incidence of an increase in in-vitro thrombotic tendency in patients with abnormal coagulation profiles, and whether this was associated with an increased risk of subsequent thromboembolic events and a reduced risk of allogeneic transfusion.

Methods:

Critically ill patients with at least one of following criteria, platelet count $<150 \times 10^9/L$, International Normalized Ratio >1.5 , or activated Partial Thromboplastin Time $>40s$, were recruited into the study. A single whole blood sample was obtained from each patient for thromboelastograph (TEG[®]) testing; and a maximum-amplitude on the TEG[®] heparinise channel $>72mm$ was defined as an increase in in-vitro thrombotic tendency. Patients with malignancy, bone marrow or hereditary coagulation disorders were excluded and the treating clinicians were blinded to the TEG[®] results to avoid in a change in clinical management of the patients.

Results:

Of the 215 patients included in the study, 37 patients (17.2%, 95% confidence interval [CI]: 12.8-22.8) had an increase in in-vitro thrombotic tendency which was associated with both a higher risk of subsequent thromboembolism (35.1% vs. 6.7% in those without thrombotic tendency, odds ratio [OR]: 7.5, 95% CI: 3.1-18.3; $p < 0.001$) and a reduced risk of requiring allogeneic blood product transfusion (13.5% vs. 43.8% in those without thrombotic tendency, OR: 0.2, 95% CI: 0.1-0.5, $p = 0.001$). Platelet count and fibrinogen concentration were the only two coagulation parameters significantly associated with the occurrence of an increase in in-vitro thrombotic tendency.

Conclusion:

An increase in in-vitro thrombotic tendency was not rare, and this was associated with a higher risk of subsequent thromboembolic events in patients with abnormal coagulation parameters. Randomised controlled trials are needed to determine whether using in-vitro thrombotic tendency to guide selective anticoagulant prophylaxis can improve patient outcomes.

418. Novel treatments in haemophilia

Peyvandi F
University of Milan

Abstract not provided

419. I'm a gene in a bottle, baby: the beginning of the end of haemophilia?

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It is an exciting time for genetic therapies. Since 1989 over 1500 Phase I/II studies of direct *in vivo* and cell-mediated gene therapy in diverse diseases have been completed (1). Substantial evidence now exists that demonstrates improved clinical outcomes in haemophilia B, immune deficiencies, haemoglobinopathies, immunotherapies for cancer and blindness. For two decades our team has participated in the basic and clinical research needed to bring investigator- and industry-sponsored gene therapy for haemophilia to fruition. Although they persist to a lesser degree, the early obstacles involving lack of clinically meaningful gene transfer efficiency and unexpected immune limitations have been addressed. Ethical concerns including inadvertent germ line gene modification have been widely considered in academic circles, yet they remain predominately represented publically in dystopian science fiction terms (2).

The current generation of patients with haemophilia B takes for granted regular venipuncture and IV administration of either plasma-derived or recombinant FIX protein replacement on-demand or as prophylaxis. It is possible now to anticipate a future where select cases could be treated by a single IV infusion of AAV-based gene therapy.

1. Rasko JEJ. A Gene Therapy Renaissance? *J Gastroenterology/Hepatology*, May;25(5):848-50, 2010; Macpherson & Rasko, Cellular therapy in the Asia-Pacific region, *Pathology*, 2011, 43(6), 616–26
2. Rasko JEJ, O'Sullivan G, Ankeny RA. Editors. *Ethics of Inheritable Genetic Modification: A Dividing Line?* 2006, Cambridge University Press

420. Current haemophilia management in Australia: Lessons learnt from the Bleeding Disorder Registry

McRae S

Abstract not provided

421. Management of immune thrombocytopenia - guided by symptoms of bleeding or platelet count

Pabinger I

Medical University Vienna

Immunthrombozytopenia (ITP) has very diverse clinical presentations. On the one hand there is the completely asymptomatic patient with decreased platelet count and the diagnosis is based just on the laboratory result and not on symptoms, on the other hand there is the patient with a severe bleeding tendency and even life-threatening bleeding, who does not respond to treatment. In addition to that, ITP is also a pro-thrombotic disorder with an increased risk of arterial and venous thrombosis and thromboembolism. In some cases both is seen at the same time: bleeding and thrombosis or even pulmonary embolism. Hematologists caring for ITP patients are in the dilemma, whom to treat, when to treat and how to treat. The bleeding risk is relatively low in young patients, which tolerate platelet counts of less than 10 G/l often with just minor and sometimes even without any bleeding symptoms. On the other hand elderly patients have a definitely increased risk of life-threatening bleedings, such as cerebral bleeding. In the recent years, guidelines have opened an individualized approach for ITP management. There is agreement, that patients with a WHO bleeding score grade 3 or 4 have to be treated irrespective of the platelet count, in most patients with WHO score grade 2 many hematologists will see an indication for treatment. The other situations are patients with very low platelet count below 10 or even 5 G/l, with only very mild bleeding manifestations. I tend to offer treatment to those with a WHO bleeding score of less than 2 in young, when their platelet count is less than 5 to 10 G/l and in elderly patients, if their platelet count is less than 20 to 30 G/l, based on the age associated increased bleeding risk.

Research is needed specifically for prediction of severe bleeding and response to treatment. For patients, which are refractory to the presently available treatments, we need novel drugs and new therapeutic concepts.

422. Integrated pathways for investigating and managing patients with platelet disorders

Rabbolini D

Royal North Shore Hospital

Inherited platelet disorders comprise a heterogeneous group of mucocutaneous bleeding conditions characterised by qualitative platelet functional defects (IPFD) and/or low platelet counts (Inherited platelet number disorders (IPND)/ inherited thrombocytopenia). The prevalence of IPFD and IPND are probably underestimated due to under-diagnosis. Accurate diagnosis is important to enable proper genetic counselling, surveillance and guide treatment strategies. Moreover, several disorders predispose individuals to extra-haematological complications such as chronic kidney disease, cataracts and sensori-neural hearing loss in the case of *MYH9*-related disorders causing macrothrombocytopenia with associated döhle-like bodies. Whilst, conditions associated with mutations in *ETV6*, *ANKRD26*, *RUNX1* are associated with solid organ and haematological malignancies, including acute myeloid and acute lymphoblastic leukaemia.

Diagnosis is generally employed in a step-wise fashion. Using this approach for IPFDs, first and second tier tests interrogating platelet functional pathways may provide a diagnosis or raise diagnostic suspicion in 40-60%, indicating that a large proportion of patients are without diagnosis even to a pathway level. Increasing access to genetic testing in tertiary centres has increased the number of IPFDs and IPNDs with identifiable genetic causes. However even with these high throughput genome screening approaches the sequence variation may still not be identified or may be difficult to prove as the causative mutation.

This presentation will provide an updated guide to the diagnosis of IPFD and IPNDs highlighting the importance of an approach which integrates phenotypic and genetic information and will discuss available treatment options.

423. Thrombosis management in the bleeding patient

Merriman E

Royal North Shore Hospital

Patients with recent thrombosis or at high risk for thrombosis who are bleeding/at high risk of bleeding are challenging, and evidence-based guidance regarding management of these patients is often lacking. Illustrative cases studies will be reviewed, and management discussed, with cases ranging from recent intracranial haemorrhage through to patients with moderate to severe thrombocytopenia and bleeding disorders.

424. Global coagulation assay changes in haemodialysis patients

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Aim:

Chronic kidney disease (CKD), and haemodialysis in particular, is thought to be a hypercoagulable state, which may contribute to increased cardiovascular risks. Given the limitations of current available coagulation testing in assessing thrombotic risks, we aim to evaluate the changes of global coagulation assays in patients with CKD undergoing haemodialysis.

Method:

This prospective observational study recruited patients undergoing haemodialysis. Fasting blood samples were collected prior to starting haemodialysis for baseline investigations such as full blood evaluation, coagulation studies and lipid studies, in addition to experimental testing via thromboelastography (TEG® 5000S) utilising citrated whole blood. Additional samples were double-centrifuged to obtain platelet-poor plasma for later assessment with calibrated automated thrombogram (CAT) and overall haemostasis potential (OHP).

Results:

Twenty-one patients were recruited and the results were compared to age-matched normal controls previously collected. Haemodialysis patients had lower platelet count with increased fibrinogen, VWF antigen and factor VIII levels ($p < 0.01$). They also had more prothrombotic TEG® profile when compared to normal controls, with increased maximal amplitude (71.1 vs 60.0 mm, $p < 0.001$) and reduced clot lysis (0.0% vs 0.5%, $p = 0.001$). Interestingly, there was no significant difference in the thrombin generation parameters. In addition, D-dimer was markedly increased in haemodialysis patients independent of age with only 4 patients found to be within normal range (770 vs 189 ng/mL, $p < 0.001$) but this did not correlate with fibrin generation parameters.

Conclusion:

Haemodialysis patients appear to have a prothrombotic state characterised by increased fibrinogen, VWF antigen and factor VIII levels, as well as TEG parameters. D-dimer was markedly increased, which brings into question the clinical usefulness of D-dimer, in predicting venous thromboembolism, in haemodialysis patients. The lack of correlation with the fibrin generation assay may signify reduced renal clearance of D-dimer. Further investigation utilising pre- and post-dialysis serum and urine D-dimer may help to confirm this.

425. Fluid resuscitation with 0.9% saline induces haemostatic changes in an ovine model of endotoxemic shock

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Aim:

Sepsis is a leading cause of mortality and critical illness and is associated with abnormalities in coagulation ranging from mild symptoms to fulminant disseminated intravascular coagulation (DIC). Fluid resuscitation is one of the foundations for the acute management of sepsis, however, there are many uncertainties surrounding the type and volume of fluid that is administered. We used an ovine model of endotoxemic shock to determine if fluid resuscitation with saline results in haemostatic derangements.

Method:

Sheep were randomly divided into no endotoxemia (control) (n=5) or endotoxemia groups (n=16) with an escalating dose of lipopolysaccharide (LPS) up to 4 µg/kg/hr. Endotoxemia sheep received either no saline resuscitation (n=8) or a 0.9% saline bolus (40 mL/kg over 60 minutes) (n=8). Control animals underwent fluid resuscitation with a 0.9% bolus of saline. Arterial blood samples were collected at baseline, pre LPS infusion (or control), post endotoxemia/pre resuscitation and post saline resuscitation at 0, 1, 3, 6, 9 and 12 hours for full blood count and ROTEM[®] analysis.

Result:

Endotoxemia animals had decreased platelet counts ($p < 0.05$) compared to controls. Prolonged contact activated (INTEM) and tissue factor activated (EXTEM) clotting time (CT) ($p < 0.05$) and clot formation time (CFT) ($p < 0.001$) was apparent, with a corresponding reduction in maximum clot firmness (MCF) ($p < 0.001$) in endotoxemia groups compared to controls. These parameters were further protracted in endotoxemia + saline animals 9hrs post resuscitation with the addition of a prolonged FIBTEM-CT ($p < 0.001$).

Conclusion:

Endotoxemia impairs secondary haemostasis and induces changes in the intrinsic and extrinsic pathways that is exacerbated 9 hours post saline resuscitation. These changes in haemostasis may put patients at risk of both bleeding and thrombotic complications several hours after initial resuscitation is given.

426. rVIII-SingleChain in surgical prophylaxis: efficacy and safety in 35 surgeries

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Background:

rVIII-SingleChain, a novel recombinant Factor VIII, was designed as a B-domain truncated construct with a covalent bond between heavy and light chains, resulting in high binding affinity to von Willebrand Factor.

Aims: To investigate the safety and efficacy of rVIII-SingleChain to control haemostasis in paediatric, adolescent and adult patients with severe haemophilia A undergoing surgery.

Methods:

Studies in the AFFINITY program were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki. In the surgical substudies, 28 patients underwent 35 procedures requiring general, spinal or regional anaesthesia. Dosing was guided by WFH recommendations. rVIII-SingleChain was used either as a bolus or continuous infusion. Haemostatic efficacy of rVIII-SingleChain during surgery was rated by investigators.

Results:

Surgical procedures performed were: abdominal hernia repair, ankle arthroplasty, ankle hardware removal, appendectomy, arthrodesis of the ankle joint, cholecystectomy, circumcision (9), debridement (2), elbow replacement, excision curettage and bone grafting, dental extraction (3), knee arthroscopy, knee replacement (7), knee spacer and immobilization, lengthening of achilles ligament (2), open reduction and internal fixation of ankle and port-a-cath removal. 27 surgeries were performed using bolus infusion while 8 surgeries used continuous infusion of rVIII-SingleChain. In 32 (91%) procedures efficacy was rated as excellent (defined as haemostasis not clinically significant different from normal) and in 3 (9%) surgeries efficacy was rated as good (defined as haemostasis normal or mildly abnormal in terms of quantity and/or quality eg, slight oozing). No related AEs or SAEs were observed during the surgery period.

Conclusion:

rVIII-SingleChain provides very effective and safe control of haemostasis during a wide range of surgical procedures when dosed either by bolus or continuous infusion.

427. Using innovation to standardise and optimise acute deep vein thrombosis (DVT) management

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Aim: Patients in Westmead Emergency Department (ED) were receiving care for acute DVT with marked practice variability. Our aim was that by June 2017, 95% of acute DVT patients presenting to ED would undergo standardised assessment, investigation, treatment initiation and follow up consistent with evidence-based best practice guidelines.¹

Method: Using quality improvement methodology, problem drivers were identified and a range of solutions devised and implemented (Table 1). A web-based clinical decision support tool (CDST) was developed using agile user methodology. Outcome, process and balancing measures of project success were evaluated by conducting audits, staff and patient surveys and analysing CDST user behaviour.

Results: 92% and 100% patients received DVT diagnosis and treatment information in ED. Patients who had all baseline tests increased from 58% to 83%. Patients reported reduced burden from their anticoagulant treatment, the median number of treatments received reduced from 2 to 1 and patients requiring therapy change reduced from 46% to 18%. Enabling patient access to the most appropriate therapy in ED resulted in reduced hospital in the home (HITH) utilization by 48%. Improved referral processes resulted in 92% patients being seen for follow-up within 3 days of discharge from ED. Balancing measures included an increase in proportion of patients seen by Thrombosis subspecialists for follow-up from 68% to 94%. Self-reported adverse events occurring prior to clinic follow-up reduced from 22% to 12% of patients. Overall cost savings of \$34058.91 were made in the first 4 months of project implementation. Overall project outcome was an increase from 13% to 84% in patients who underwent all appropriate investigations, treatment and follow-up with a reduction in cost per patient from \$1985 to \$1042.

Conclusion: Streamlining processes for investigation, treatment and referral of acute DVT patients, supported by an innovative CDST resulted in improved efficiency, standardisation and significant cost savings.

Reference: 1. Kearon C, Akl EA, Ornelas J et al, Antithrombotic Therapy for VTE disease, CHEST guideline and expert panel report. Chest. 2016; 149(2) 315-52.

Table 1: Solutions to standardise care for patients presenting to ED with acute DVT

No.	Solution
1	Web-based, mobile friendly clinical decision support tool (CDST) to assist clinicians in assessment, diagnosis and treatment of DVTs
2	Standardised pathology order set on Powerchart
3	One point of contact for Haematology advice on DVT
4	Establishment of rapid access Acute Clot Clinic (ACC) outpatient appointments
5	e-Referral system for Acute Clot Clinic (ACC)
6	Provision of NOAC starter packs and development of a process for 24 hour dispensing from ED
7	Patient information leaflets developed and provided with all starter packs
8	Multi-disciplinary education sessions for ED and Haematology staff on best practice, new model of care and treatment modalities for patient cohort.

428. Real-world experience of direct oral anticoagulants use in North Melbourne, Australia

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Aim: Direct oral anticoagulant (DOAC) is increasing used in the management of atrial fibrillation (AF) and venous thromboembolism (VTE). There is limited local data evaluating the real-world use of DOACs, particularly compliance to prescribing recommendations and safety data. We aim to evaluate our real-world DOAC usage, in comparison to clinical trials and other reported real-world experience.

Method: Retrospective evaluation of all patients commencing/continuing on DOAC between September 2013 and June 2016, through Northern Health. Data collected included demographics, duration of therapy and safety outcomes.

Result: 884 patients with median age 69 years (17-96) and 50.3% (n=445) males were identified. The average creatinine clearance (CrCl) at commencement of therapy was 85.9 mL/min, with 10 patients having CrCl <30mL/min. The most common indication for DOAC was AF (n=523, 59.1%) with average CHADS2VASc score of 4. The most common prescription for AF is Rivaroxaban 20mg daily (35.9%) followed by apixaban 2.5mg twice daily (n=117, 22.4%). The remaining indications were for VTE treatment (n=277) and maintenance or thromboprophylaxis (n=83). The majority of patients were initially treated with Rivaroxaban (96.4%) with average duration of anticoagulation of 5.2 months. Overall, there were 27 episodes of clinically significant bleeding (ISTH-SSC score 3/4) including one resulting in death. These patients were more likely to be older (median age 78, p=0.02) and 6 patients (22.2%) were on concurrent anti-platelet therapy, compared to 13.2% in the non-bleeders (p=0.18). Table 1 compares our bleeding rates to those reported by the clinical trials and other real-world studies. In terms of recurrent thrombotic complications, there were 10 episodes of ischaemic stroke (1.9%) and 9 episodes of VTE (2.5%) while on DOACs.

Conclusion: The bleeding and recurrent thrombosis rate in our study is comparable to real-world and clinical trial data although interestingly, it was higher in AF patients on apixaban.

AF (n=523)	Real Life Data for AF		Clinical Trial Data for AF		
DOAC	<i>This study</i>	<i>Danish study (Larsen et al)</i>	<i>ROCKET -AF</i>	<i>RELY</i>	<i>ARISTOTLE</i>
Rivaroxaban(45.5%)	2.1% (5/238)	3.6%	3.6%		
Dabigatran (17.2%)	3.3% (3/90)	2.0%		2.9-3.3%	
Apixaban (37.3%)	6.2% (12/195)	2.7%			2.1%
VTE (n=361)	Real Life Data for VTE		Clinical Trial Data for VTE		
	<i>This study</i>	<i>XALIA</i>	<i>EINSTEIN</i>		<i>AMPLIFY</i>
Rivaroxaban (90.0%)	2.2% (7/325)	0.7%	8.1% (including clinically relevant non-major bleeding)		
Apixaban (9.7%)	0% (0/35)				0.6%

429. Successful treatment of Alemtuzumab induced ITP with a thrombopoietin receptor agonist

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Introduction:

Alemtuzumab is a humanised monoclonal antibody targeting CD52, and achieved TGA approval in 2013 for relapsing remitting multiple sclerosis (rrMS) after rebranding as “Lemtrada”. The association between alemtuzumab and autoimmune disease including a fatal case of ITP is now well established. The use of thrombopoietin receptor agonist (TPO-RA) for alemtuzumab associated ITP has not been previously reported.

Method:

We describe a 47 year old female treated with alemtuzumab for rrMS. 23 months following the use of alemtuzumab treating her rrMS, our patient developed a steroid responsive neutropenia followed by thrombocytopenia with a platelet count nadir of $10 \times 10^9/L$. She was treated with steroids/IVIG. She experienced numerous treatment related complications. The durability of platelet responses to IVIG diminished, and prednisone doses could not be sustained below 20mg daily. Second line treatment with splenectomy and rituximab were not considered suitable. She was JC virus positive. She failed dapsone treatment and was treated with eltrombopag (patients TPO choice). She also developed Graves disease and was treated with carbimazole and radioactive iodine.

Results:

The patient attained a complete response with eltrombopag, permitting steroid/IVIG cessation soon after. Following dosing guidelines, she relapsed with bleeding and platelet nadir of $5 \times 10^9/L$. She was salvaged successfully with eltrombopag on which she currently remains at a dose of only 25mg every third day.

Conclusion:

This case highlights a rare but reported autoimmune complication of an emerging therapy for rrMS. This represents the first known report of successful treatment in Alemtuzumab-induced ITP with a TPO-RA. Clustering of thyroid autoimmunity and thrombocytopenia may reflect the long observed association between Graves disease and ITP in which HLA-B8 genotypes may participate, and the predictable timing of these complications after Alemtuzumab offers an insight into the pathogenesis of autoimmune disease. TPO-RAs may be safe and effective for Alemtuzumab induced ITP.

433. Simultaneous measurement of platelet aggregation and platelet-leucocyte conjugation using small volume of fixed whole blood

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Introduction:

The current platelet function testing is time and labour-intensive, requires a significant volume of blood sample which has to be processed within a limited time after venepuncture. We have developed a method to assess platelet aggregation and platelet-leucocyte conjugate formation in fixed whole blood samples. Here we evaluated this assay performed in the 96-well plate format which offers the advantage of using a reduced blood volume.

Methods:

Platelet function was assessed in whole blood obtained from healthy volunteers, using 96-well plates coated with 4µl of arachidonic acid, ADP, collagen and TRAP, or vehicle. Whole blood was also incubated with aspirin, cangrelor or their combination. 46µl of whole blood was added to each well and the plate was shaken for 5min at 1000 rpm at 37°C; a fixative solution AGGFix (Platelet Solutions Ltd., Nottingham, UK) was applied to stop platelet aggregation and stabilise samples for up to 9 days prior to analyses; aggregation was assessed by flow cytometry as a decrease in the number of single platelets. Platelet-leucocyte conjugates analysis in the same whole blood samples was completed within 3 days after fixation.

Results:

In 10 healthy volunteers aggregation assessed in duplicate was robust and reproducible (CV<10%). As expected, cangrelor induced a profound inhibition of ADP-induced aggregation and aspirin dose-dependently inhibited platelet responses to AA. Collagen- and TRAP-induced aggregation was also impaired to a different extent by *in vitro* treatment with either antiplatelet agent. Platelet-leucocyte conjugate formation was readily measured in the same whole blood samples and was reduced by platelet inhibition.

Conclusions:

Platelet aggregation and conjugate formation assessed by flow cytometry in fixed whole blood might be useful for investigation of platelet function, especially in individuals where blood sample volume is limited. Further investigation to determine the clinical utility of the assay in detecting acquired and inherited defects in platelet function is warranted.

434. Platelet IL-1 β release in response to ADP and epinephrine appears to be microtubule-dependent and caspase-1-independent

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The circulating pro-inflammatory cytokine IL-1 β is upregulated in many diseases and chronic inflammatory states including coronary artery disease, rheumatoid arthritis and diabetes mellitus; in all these there is increased risk of thromboembolism. The exact mechanism involved in platelet IL-1 β regulation/release is uncertain and appears to be agonist dependent. So far the suggested mechanisms have included caspase-1 and β 3 integrin dependence in washed platelets (1, 2).

Aim: We aim to examine whether caspase-1 is involved in the release of IL-1 β from platelets after stimulation with ADP and epinephrine and to determine if microtubule inhibition is involved.

Method/Results: Platelets from CAD patients express increased NLRP3 (intracellular flow cytometry) compared with healthy controls (70.8 \pm 14.3, n=8 vs 45.2 \pm 6.7 MFI, n=5), and higher plasma levels of IL-1 β (ELISA) than controls (17.5 \pm 14.3, n=10 vs 8.5 \pm 4.3 pg/mL, n=4). Platelets (from platelet rich plasma) were stimulated with ADP, epinephrine or LPS+ATP for 2.5 hours. This led to a significant increase in plasma levels of IL-1 β , however, LPS+ATP stimulation did not (classical stimulation in leukocytes); this upregulation appears to be caspase-1 independent (Figure 1B, n=4). The LPS+ATP and caspase-1 inhibitor were confirmed to work in macrophage culture for IL-1 β secretion and inhibition. The observed upregulation was decreased with microtubule inhibition doses of colchicine (2 mM) by 35% and 49% for ADP and epinephrine stimulation respectively (n=3).

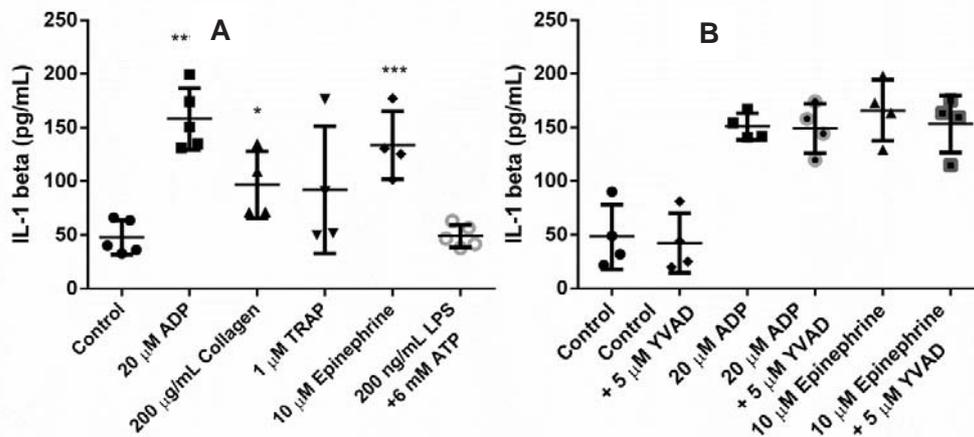


Figure 1: IL-1 β secretion in PRP in response to ADP, collagen and epinephrine (A) and caspase-1 inhibition with Z-YVAD-FMK (B) did not lead to decreased IL-1 β . Mean \pm SD, paired t-test with control, P<0.05, n=4-5.

Conclusion: Platelet IL-1 β release in response to stimulation with ADP and epinephrine appears to be microtubule dependent and caspase-1 independent in the setting of platelet rich plasma. Further caspase inhibitors need to be assessed. Platelet IL-1 β release is differential in regulation to leukocytes.

1. G. T. Brown *et al.*, *J Immunol* **186**, 5489 (2011); 2. S. Lindemann *et al.*, *The Journal of cell biology* **154**, 485 (2001).

435. Altering the splicing of the F8 mRNA transcript as a future treatment for haemophilia A

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Aim:

Investigate the feasibility of inducing complete or partial skipping of exon 14 from the *F8* mRNA transcript using 2' O-methyl phosphorothioate antisense oligonucleotides (AOs) as a possible future therapeutic treatment option for haemophilia A patients with mutations mapping to the large B-domain of FVIII.

Method:

HuH-7 cells were transfected with a range of AO sequences specific to predicted *F8* exon 14 splice site recognition features. Following transfection, total RNA was harvested, converted into cDNA and PCR performed using primer sequences spanning the exon 13/14 and exon 14/15 boundaries. Truncated PCR products were isolated, sequenced and compared to the wild-type sequence to determine if the modified sequence retained an in-frame codon usage. 2'-O-MeAOs demonstrated to effectively modify exon 14 whilst maintaining correct codon usage will be used for FVIII quantitation and functional analyses using a FVIII ELISA and two-step chromogenic assay.

Results:

HuH-7 cells were successfully transfected with a number of AOs, both in isolation and in combination and preliminary data suggests that some AOs can induce complete skipping of exon 14, while others cause a partial removal of exon 14 sequence. Further analysis is ongoing to determine the exact splicing that has occurred and whether or not the transcripts retain a correct, in-frame codon usage.

Conclusion:

The preliminary data from this study suggests that skipping of exon 14 that encodes for the large B-domain of FVIII is feasible. The B-domain is removed from the wild-type FVIII molecule as part of normal post-translational processing and is not required for aFIX cofactor function; many recombinant forms of FVIII are defined as B-domain deleted. Therefore, development of technology shown to induce skipping of *F8* exon 14 provides a potential future therapeutic option for haemophilia A patients with mutations mapping to exon 14 of their *F8* gene.

Conflict of Interest:

No conflict of interest to disclose.

436. Compare out come of out patient Vs Inpatient management of pulmonary embolism in peninsula health

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Background:

Pulmonary embolism (PE) is a common life threatening cardiovascular condition, with 112 incidents per 100,000 per year (1). Outpatient treatment instead of traditional inpatient treatment in selected low – risk patients with acute PE might provide several advantages such as reduction of hospital admissions, substantial cost saving.

Objective:

To compare the efficacy and safety of outpatient versus inpatient treatment for acute PE.

Method:

All patients who presented to Frankston Hospital Emergency Department (ED) with acute PE, confirmed by Computed Tomography Pulmonary Angiogram (CTPA) or Ventilation/Perfusion (VQ) scan, from January 2015 to June 30, 2016 were included in the study. Age, sex, severity of PE, simplified PESI score, type of anticoagulant used and duration of hospital stay were recorded for those selected. Outcome related data, gathered at 3 months post treatment, included mortality, bleeding manifestations requiring admission and other complications.

Results:

A total of 177 patients met the inclusion criteria for image proven acute PE. Of those 108 were managed as inpatients and 69 were discharged on anticoagulation from ED. Six (5.5%) deaths were reported in the inpatients group and 3(4.34%) reported in the outpatients group. No deaths were directly related to PE or anticoagulation in both groups. Eight (7.4%) from the inpatients group were readmitted less than 3 months after discharge while six (8.69%) from the outpatients group needed readmission. Three (2.7%) patients in the inpatients group and two (2.8%) patients in the outpatients group needed hospital admission due to bleeding during the first 3 months.

Discussion:

This study shows similar outcomes in all cause mortality, readmission, significant bleeding and other complications in those receiving inpatient vs outpatient anticoagulation. These findings suggest that, patients with low risk (Low PESI) can be discharged from ED after commencing the most appropriate anticoagulation safely with follow up by general practitioner and the haematologist.

Ref

(1)- Wiener RS, Schwartz LM, Woloshin S. Time trends in pulmonary embolism in the United States: evidence of over diagnosis. Arch Intern Med 2011; 171:831

437. Evaluation of global coagulation assay parameters in normal and thrombocytopenic populations

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Aim: Quantitative platelet count may not reflect the bleeding risk and routine coagulation testing only assesses time to initial clot formation. Global coagulation assays such as thromboelastography (TEG) and thrombin generation with calibrated automated thrombogram (CAT) may provide a more complete assessment of an individual's coagulation profile. We aim to evaluate how global coagulation assays differ between thrombocytopenic patients and normal controls.

Method: Blood samples were collected from patients with platelet $150 \times 10^9/L$ for baseline laboratory investigations and global coagulation assays. Citrated whole blood was analysed using TEG® 5000 analyser and CAT was performed on platelet-poor plasma. The results were compared to our previously collected normal control population.

Result: Fifty-eight samples (30 males, 28 females; mean age 57.5 years; 24 immune thrombocytopenia/other causes, 34 chemotherapy/malignancy-related thrombocytopenia) were collected. Thrombocytopenic participants (average platelet $79 \times 10^9/L$) had reduced clot lysis (0.0% vs 0.6%; $p < 0.001$) on TEG® without significant difference in clot strength (maximum amplitude, 59.3 vs 57.8 mm; $p = 0.38$) while CAT showed reduced endogenous thrombin potential (1252.2 vs 1353.0 nM.min; $p = 0.040$). On sub-analysis, participants with marked thrombocytopenia ($0-50 \times 10^9/L$) had prolonged clot formation time (K-time, 3.4 vs 2.3 min; $p = 0.002$) with reduced α -angle (41.2° vs 57.9° ; $p = 0.001$), clot strength (47.2 vs 57.8 nM.min; $p = 0.002$) and lysis (0.0% vs 0.6%; $p < 0.001$). Conversely, participants with platelet count $100-150 \times 10^9/L$ had reduced clot formation time (1.7 vs 2.3 min; $p < 0.001$) and increased clot strength (66.4 vs 57.8 nM.min; $p < 0.001$) compared to normal controls. No significant difference between the ITP and chemotherapy/malignancy patients was observed apart from reduced clot lysis in the latter ($p = 0.018$)

Conclusion: Marked thrombocytopenia ($< 50 \times 10^9/L$) showed hypocoagulable parameters, which validate current clinical practice of managing this population as high bleeding risk. Interestingly, lysis was markedly reduced in thrombocytopenic population, suggesting compensatory mechanisms to reduce bleeding risk. Larger prospective studies will be undertaken to confirm these findings.

438. Challenges in TTP Prognostication Using ADAMTS13 Activity and Inhibitor Assays - APMAT Network Study Report

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Aim: An ADAMTS13 level of <10% in patients with microangiopathic thrombocytopenia (MAT) currently confirms a diagnosis of thrombotic thrombocytopenic purpura (TTP) and differentiates TTP from other MAT causes. The APMAT Network implemented a standardised ADAMTS13 testing in key Asia Pacific laboratories utilising controlled assays kits, coded and well characterised plasmas samples; combined with an external quality assessment study of assays performed at these centres. The study aims to establish and assess standardised ADAMTS13 testing in the APMAT Network and examine any sample classification variabilities.

Methods: Two surveys were conducted 6 months apart at 23 laboratories across 10 countries (Australia, New Zealand, China, Korea, Japan, Taiwan, Hong-Kong, Malaysia, Thailand, India and Singapore). Each survey utilised four coded plasma samples of known activity and inhibitor levels. Participating laboratories performed ADAMTS13 activity and inhibitor titre assays using the same chromogenic ELISA format (Technoclone); the results were compared to ECAT cohorts.

Results: The APMAT survey sample mean and standard deviation were slightly higher when compared to the ECAT cohort, but still within the expected variation (<20%). Inter-laboratory variations for activity samples (mean, CV%) were: APM01(0.2%,289), APM02(17%,29), APM05(6.5%,40), APM06(95.5%,14) and for inhibitor samples APM03(5U/ml, 35), APM04(11U/ml,18), APM07(7U/ml,31), APM08(14U/ml,20). ADAMTS13 inhibitor survey results contained more outliers (5-10%). For activity and inhibitor assays, 90% and 84% of laboratories achieved a passing z-score ($-2 < z\text{-score} < 2$), respectively. For controls (intra-laboratory measure), 100% and 95% were within the pass z-scores for activity and inhibitor assay, respectively. Sample classification is clear for high and low ADAMTS13 levels, but ambiguous for the current cut-off of 10%. Inhibitor levels gave a heterogeneous classification pattern.

Conclusions: ADAMTS13 assay QC is feasible in an international multicentre setting and has potential to expedite correct MAT diagnosis at local centres. Classification challenges remain for activity levels proximal to the current 10% cut-off; inhibitor alone is insufficient for TTP classification.

439. Congenital thrombotic thrombocytopenic purpura in pregnancy, a case report

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Introduction:

Congenital Thrombotic Thrombocytopenic Purpura (TTP) is a rare inherited deficiency of ADAMTS-13 (A Disintegrin And Metalloproteinase with Thrombospondin type-1 motif, member 13) that is classically diagnosed in neonates and children but rarely presents in adults. Pregnancy is a potential trigger for undiagnosed congenital TTP. First presentations of congenital TTP in pregnancy are associated with a high maternal morbidity and foetal mortality.

Case Report:

A 27 year old woman presented with a severe thrombocytopenia and red cell fragmentation at 20 weeks gestation. She was diagnosed with TTP and treated with plasma exchange and steroids. Despite an improvement in her blood counts with treatment she developed severe pre-eclampsia with a marked proteinuria and difficult to control blood pressure. The foetus demonstrated intrauterine growth restriction (IUGR) and the patient had a still birth at 23 weeks gestation. Post-partum ADAMTS-13 testing demonstrated reduced levels (6-8%) without an inhibitor, consistent with congenital TTP. ADAMTS-13 gene mutation studies were indeterminate.

The high risk nature of subsequent pregnancies was discussed with the patient and she elected to proceed with another pregnancy. A strict regime of full blood count monitoring with regular plasma infusions to maintain a platelet count in the normal range was instituted. Her second pregnancy was complicated catheter associated DVT, gestational diabetes and preeclampsia. Due to the severity of the preeclampsia and the development IUGR a caesarean was performed at 33 weeks with the delivery of a live singleton.

Conclusion:

While congenital TTP triggered by pregnancy is often associated with significant morbidity in the presenting episode, subsequent pregnancies may be managed with blood product support in an appropriate setting to achieve successful outcomes.

440. Toxicity profile of Vitamin K Antagonist and the management strategies

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Aim:

Vitamin K Antagonists (VKA) are one of the widely used anticoagulant. Patients on long-term therapy have a risk of 1%–3% per year for hemorrhage leading to hospitalization or death. Despite the associated bleeding risk, warfarin remains the most commonly prescribed anticoagulant in many parts of the world primarily because of easy availability and cost-effectiveness. The aim of the study was to determine the frequency of hemorrhagic complications in patients on warfarin. The secondary objective was to assess their risk factors and to evaluate the management strategies given in warfarin toxicity

Method:

It was a retrospective study. All patients on warfarin who presented with bleeding complications from January - December 2016 were included in the study. Age, gender, indications, risk factors for bleeding, severity and management were reviewed from patient's record. Data was recorded on a predesigned proforma.

Result:

The cohort included 51 patients (mean age 59 +/- 10 years, 64% male). Majority of patients were taking oral anticoagulant for therapeutic purpose most common being Atrial fibrillation (n=32, 63%) followed by thromboembolism (n=11, 21%) and prosthetic heart valves (n=3, 06%). Common risk factors included old age (n=31, 60%) and hypertension (n=22, 43%). Major bleeding as per ISTH criteria was observed in 13 individuals (25.4%). Most common site of bleeding was gastrointestinal (n=27, 52.9%) followed by hematuria (n=13, 25.4) and bruises (n=08, 15.6%). Intracranial bleeding leading of death was observed in one case. The drug was discontinued in all cases with transfusion of fresh frozen plasma and use of intravenous vitamin K supplementation in 33% (n=17) and 94% (n=48) cases respectively.

Conclusion:

The rate of major bleeding and fatal complication with oral anticoagulants in our population where VKA are still preferred over novel anticoagulants is low. With regular monitoring, patient education and prompt management, bleeding complications can be reduced.

443. High adherence in adult and paediatric patients with haemophilia B receiving prophylaxis with rIX-FP

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Background:

rIX-FP is a fusion protein genetically linking recombinant human coagulation Factor IX with recombinant human albumin. In clinical trials, 7-, 10- or 14-day rIX-FP prophylaxis in adults (≥ 12 years) and 7-day prophylaxis in paediatric patients (< 12 years) achieved median annualized spontaneous bleed rates of 0.00.

Aims: This analysis evaluated adherence to different regimens in two clinical trials of patients with haemophilia B (FIX $\leq 2\%$).

Methods:

Adults with haemophilia B received either on-demand treatment for 6 months then prophylaxis every 7 days (on-demand arm; n=23) or 7-day prophylaxis for 6 months then, if eligible, prophylaxis once every 10 or 14 days (prophylaxis arm; n=40). Paediatric patients (n=27) received prophylaxis every 7 days. Dose, dosing frequency and rIX-FP consumption was recorded in an e-diary; reported adherence was reconciled with the number of used vials returned at each study visit. Adherence to prophylaxis was determined in terms of schedule (defined as a compliance rate $\geq 80\%$; adults and paediatrics) and prescribed dose (adults only).

Results:

In the adult study, 94.9% of subjects were adherent to their prophylaxis schedule. Mean prophylaxis compliance rate for the 7-day regimen was similar between on-demand and prophylaxis arms (95.5% and 94.7%, respectively). Mean compliance rates with 10- and 14-day regimens was 90.7% and 97.2%, respectively. Overall, 85.7% of adult patients were dose compliant (within 10% of prescribed dose $\geq 80\%$ of the time). In the paediatric study, all patients were adherent with the weekly prophylaxis schedule; mean overall compliance rate was 97.9% and was similar between those aged 1–5 years and those aged 6–11 years.

Conclusion:

Adherence to prophylaxis schedule is essential for bleed prevention in patients with haemophilia. Data show that 7-, 10- and 14-day rIX-FP prophylaxis regimens result in high rates of compliance and very low bleeding rates in both adult and paediatric patient populations.

444. Response of FVIII and VWF to desmopressin in haemophilia A and von Willebrand disease patients

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Aim:

Desmopressin (DDAVP) is used to release endogenous stores of Von Willebrand Factor (VWF) and hence increase both VWF antigen and Factor VIII levels with proven efficacy in both haemophilia A and Von Willebrand Disease (VWD). Efficacy has been seen at various dose levels. Side effects of DDAVP can be dose dependent and there is increasing literature regarding the venous thromboembolic risks of high Factor VIII levels. Therefore we aim to review the dosing of DDAVP as assessed by factor levels achieved during the commonly performed DDAVP trial.

Methods:

Multicentre retrospective review of pathology data performed between October 1996 and May 2015.

Results:

Seventy-eight patients received a DDAVP challenge (60 with VWD, 16 with mild haemophilia A and 2 with combined VWD and mild haemophilia A). The DDAVP dose range for VWD patients was 0.15mcg/kg to 0.36mcg/kg, mild haemophilia A patients was 0.25mcg/kg to 0.29mcg/kg and the combined patients was 0.14mcg/kg to 0.26mcg/kg. Sixty-eight patients had complete data to determine a response to the challenge. Overall 63 patient (92.6%) achieved a complete response (48 VWD, 13 haemophilia A, 2 combined group), 1 patient (1.5%) achieved a partial response (VWD) and 4 patients (5.9%) achieved incomplete response (2 VWD, 2 haemophilia A). Further analysis is pending.

Conclusion:

DDAVP is very effective in patients with VWD and haemophilia A with responses achieved through most dose levels. Doses less than the recommended 0.3ug/kg is effective and safe.

445. Von Willebrand levels in pregnancy: the predictive value of the prepregnancy response to DDAVP

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Background:

Pregnancy in von Willebrand disease (vWD) may carry a risk of significant bleeding. Successful management of pregnancy in patients with vWD requires coagulation laboratory monitoring, including response to standard treatment. A paucity of data exists regarding the predictive value of pre-pregnancy DDAVP testing.

Aim:

To investigate the predictive value of the pre-pregnancy response to DDAVP on changes in factor VIII (FVIII), von Willebrand factor (vWF) and pregnancy outcome at the Alfred Health Haemophilia Treatment Centre (AH-HTC).

Method:

Patients with vWD identified from the Australian Bleeding Disorders Registry, with pregnancy to childbirth between 2000-2017 and treated at AH-HTC were included. Only patients with pre-pregnancy laboratory information regarding DDAVP testing and adequate clinical information were included. Baseline laboratory and vWD diagnostic information was analysed. The change in FVIII and vWF levels in response to DDAVP prior to pregnancy was compared to baseline and the changes in levels throughout pregnancy. Clinical factors associated with pregnancy outcome were analysed.

Results:

19 pregnancies in 14 women met inclusion criteria for the study period. 1 woman had type 2A vWD (not demonstrating DDAVP response), the remaining 13 had type 1 vWD. 11 women (78%) demonstrated an increase to FVIII and vWF after DDAVP and during pregnancy, the changes were within 10% of the total level on each occasion. For these women, DDAVP was successfully used to manage vaginal or caesarean delivery without serious complications. Post-partum haemorrhage was uncommon (21%); use of red cell transfusion was likewise uncommon (14%). No miscarriages or stillbirths occurred.

Conclusion:

The biological response to DDAVP prior to pregnancy in women with vWD correlates well with the intrapartum changes in vWF and FVIII levels. DDAVP was effective at preventing significant bleeding in most patients demonstrating a DDAVP response.

446. DOAC levels - why we do them and do they change management?

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Aim:

Direct oral anticoagulant (DOAC) monitoring using calibrated anti-Xa assays for rivaroxaban and apixaban is available in most major Australian hospitals. Indications for its utility are unclear. We reviewed experience in our institution to assess its effect on patient management.

Method:

DOAC assays for rivaroxaban and apixaban, performed between April 2015 and September 2016, were retrieved from laboratory records. Concurrent blood results, clinical history, indication for DOAC use, time of last dose, indications for DOAC testing and effect on management were extracted from electronic records and manual case-note review.

Results:

136 assays (58% for rivaroxaban, 42% for apixaban) were performed on 95 patients [median age 71 (range 19 – 93), 63% male]. The main indication for DOAC use was atrial fibrillation (71%). DOAC levels ranged from 0.5 – 1009 ng/L. Time of last dose could not be ascertained in 35% of assays. Main indications for testing were pending surgery (47%), bleeding (18%), bleeding and pending surgery (9%), and breakthrough thrombosis (7%). Other indications included drug interactions, renal impairment, morbid obesity, suspected non-adherence, and polypharmacy overdose. 16% of assays had no clear indication for testing. Less than half of the assays (48%) changed management: 57% affected timing of surgery, 23% a decision regarding coagulation factor replacement, and 18% a decision to change anticoagulant or interacting medications. Of the 25 assays performed more than 48 hours after the last dose, mean and median DOAC levels were 43 ng/L and 23 ng/L respectively; five had levels > 60 ng/L while two had levels > 100 ng/L.

Conclusion:

DOAC testing is sometimes requested without clear indications; in many patients the test does not affect management. The main effect of DOAC testing was on the timing of surgery, and our test results suggest it may not be safe to base that decision solely on time of last dose and renal function.

447. Minimum half-life extension ratio model for reduced dosing frequency of extended half-life recombinant FVIII products

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Aim: With new recombinant factor VIII (rFVIII) products available, it is crucial to differentiate those that should be considered extended half-life (EHL) products. This supports assessment by physicians and payers and, consequently, appropriate and adequate access for patients. One criterion for defining EHL products is half-life extension ratio. However, a minimally clinically meaningful ratio has not been defined until now. This study aims to identify the half-life extension ratio required to make a clinically meaningful reduction (ie, one day) in dosing interval while maintaining the same percentage of patients consistently at a target factor VIII (FVIII) activity >1 IU/dL.

Methods: The population pharmacokinetic model for standard rFVIII (Björkman et al, Blood.2012;119(2):612-8.) was used to estimate the percentage of patients consistently >1 IU/dL using a benchmark regimen for rFVIII 3 times weekly. In a second step, dosing frequency was reduced to twice weekly and rFVIII half-life extended until the percentage of patients consistently >1 IU/dL was equal to the benchmark regimen. The result is an estimate of the minimally clinically meaningful extension ratio required to meet the definition of an EHL rFVIII product.

Results: Benchmark doses for rFVIII of 100 IU/kg/wk were tested to reflect common rFVIII use. This benchmark regimen resulted in 56.6% of patients consistently >1 IU/dL. Comparing the benchmark to doses of 80 and 90 IU/kg/wk, the fold extension required to achieve 56.6% of patients >1 IU/dL was 1.30 and 1.26, respectively (Table).

Table

Dose Regimen	Patients >1 IU/dL, %	Always	Half-Life Ratio	Extension
3 times/wk, benchmark Days 1 and 3, 30 IU/kg; day 5, 40 IU/kg	56.6		Not applicable	
2 times/wk Days 1 and 3, 40 IU/kg	56.6		1.3	
2 times/wk Days 1 and 3, 45 IU/kg	56.6		1.26	

Conclusions: Per this population pharmacokinetic model and simulation, rFVIII products should show a minimum half-life extension ratio of 1.3 for a meaningful reduction in dosing while maintaining the same percentage of patients consistently >1 IU/dL FVIII activity. Future research could investigate the extent to which EHLs that exceed the minimum extension ratio criteria may improve rFVIII coverage vs current prophylaxis regimens

448. What is the Health System Cost of Treating Haemophilia A in Australia?

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Aim:

To estimate the overall health system costs of treating severe haemophilia A (HA) in Australia using recombinant factor VIII (rFVIII), ADVATE.

Methods:

A literature-based cost study for treatment of severe HA in the absence of inhibitors was undertaken. ADVATE treatment was assumed to be given prophylactically to prevent bleeding episodes or on demand for a bleeding episode. Utilisation data for rFVIII were derived from the Australian Bleeding Disorders Registry. Total health system costs were calculated by summing the cost of rFVIII (prophylaxis and on demand), other direct medical costs for treating bleeding episodes, and any adverse events (AEs). All costs are reported in AU\$2016.

Results:

Australian data were available for five identified variables associated with treating HA: cost per IU of rFVIII; cost of some AEs; rFVIII use by haemophilia severity and by treatment regimen; and cost of orthopaedic surgeries. Australian data were unavailable for important variables, such as hospitalisations from bleeds, home therapy, orthopaedic procedures, joint abnormalities, and median annualised bleed rates, all of which are necessary to derive costs from a health system perspective. Using Australian data and supplementing international data where necessary, approximate annual health system costs for treating severe HA in the absence of inhibitors with rFVIII ADVATE was AU\$92,000 (AU\$83,000 rFVIII treatment cost) for prophylaxis and AU\$38,000 (AU\$31,000 rFVIII treatment cost) for on demand treatment per patient. The annual population cost in 2014/2015 was approximately AU\$51,612,000 (AU\$45,966,000 rFVIII treatment cost) for 640 patients with severe HA in Australia.

Conclusions:

Using Australian data supplemented with international data, the cost of rFVIII (ADVATE) is reasonable for very effective treatment in preventing and/or treating bleeding episodes and comparable to other government-funded, highly specialised, and/or life-saving drugs in Australia. Further research into the societal cost and benefits of treating HA in Australia is warranted.

449. Direct oral anticoagulant use in patients with active malignancy: evaluation of demographics and safety outcomes

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Aim:

There is limited data on the use of direct oral anticoagulants (DOACs) in patients with active malignancy particularly for the treatment of venous thromboembolism (VTE). We aim to evaluate the use of DOACs in these patients in a real-world setting, both in atrial fibrillation (AF) and VTE.

Method:

Retrospective evaluation of patients commenced or continued on DOACs while inpatient between September 2013 and June 2016 in our hospital including demographics, type of malignancy and safety outcomes.

Result:

Fifty-four patients with active malignancy on DOACs (median age 78 years; 66.7% males) were identified of which 74.1% (n=40) had solid organ malignancies and 25.9% (n=14) had haematological malignancies. Twenty-three (42.6%) patients were on DOACs for VTE-related causes and the remaining (n=31, 57.4%) were for AF (average CHADS₂VASC score 4). Sixteen patients on DOACs for VTE-related reasons had solid organ malignancies, of which nine had known distant metastasis. The median creatinine clearance (CrCl) at DOACs commencement was 66.8 mL/min (30.0-149.2). The most commonly used DOAC in VTE was Rivaroxaban 20mg daily (16/23, 69.6%) while apixaban 2.5mg twice daily was the most commonly used in AF (12/31; 38.7%). The latter group had median age of 84 years with median CrCl 40.5 mL/min. Overall, there was one episode (1.8%) of major bleeding (ISTH-SSC score 4) involving a patient with metastatic colorectal cancer on Rivaroxaban 20mg daily for VTE; and one episode of proven recurrent VTE while on rivaroxaban 20mg daily, which was comparable to our warfarin/enoxaparin era data (1/23 (4.3%) vs 9/240 (3.9%), p=0.89) recurrence on therapeutic anticoagulation). No episodes of thrombotic stroke or deaths related to DOAC use were captured.

Conclusion:

DOACs appear to be effective and safe for both AF and VTE treatment in patients with active malignancy. Larger prospective studies involving this patient population are required to confirm these results.

451. Apixaban in mechanical circulatory support – evaluation in a mock circulatory loop with human blood.

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Aim:

In light of decreased intracranial haemorrhage with apixaban compared to warfarin in atrial fibrillation trials, we conducted an *ex vivo* study of thrombus formation in an ASTM 1841 hemocompatibility testing loop incorporating a continuous flow left ventricular assist device (HeartWare HVAD). The aim of this study was to determine the effect of low and high dose apixaban on intra-pump thrombus formation.

Method:

Blood from volunteers were used in four experimental conditions: un-anticoagulated blood (control); blood from patients on warfarin; in vitro low dose apixaban (equivalent 2.5mg BID); and in vitro high dose apixaban (equivalent 5mg BID). Afterload, preload and loop temperature were controlled to physiological targets. The primary outcome was time to formation of intra-pump thrombosis based on 20% rise in power characteristics. A secondary outcome was changes in haemostasis over one hour of stable pump use as measured by microparticle activity (PPL), platelet aggregation (multiplate), rotational thromboelastometry and von Willebrand factor levels (CBA/Ag).

Result:

The overall time to intra-pump thrombosis formation was prolonged in the anticoagulated runs when compared to control (83.1vs 70.6 mins). Low dose apixaban was comparable to warfarin (80.5mins vs 80.3), however high dose apixaban showed no evidence of thrombosis after 90mins. Baseline total clotting times were prolonged in the anticoagulation groups (control 91.2s, warfarin 120s, low dose apixaban 164.4s, high dose apixaban > 180s). The reduction in clotting times over one hour pump running was similar in the control, warfarin and low dose apixaban groups (44.3s vs 37.8s vs 45.8s).

Conclusion:

In an in vitro setting, low dose apixaban provides similar anticoagulant property as warfarin in terms of prolongation of thrombosis formation. Higher dose apixaban may have lower pump thrombosis rates. Further studies in patient groups are needed before change in clinical practice.

This research was supported by a grant from the St Vincent's Clinic Foundation and from Pfizer. The company had no role in analysing the data or preparing the abstract."

452. High and sustained trough FIX activity levels with dosing of IDELVION (rFIXFP) in haemophilia B

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Background:

rVIII-SingleChain, a novel recombinant Factor VIII, was designed as a B-domain truncated construct with a covalent bond between heavy and light chains, resulting in high binding affinity to von Willebrand Factor.

Aims:

To investigate the safety and efficacy of rVIII-SingleChain to control haemostasis in paediatric, adolescent and adult patients with severe haemophilia A undergoing surgery.

Methods:

Studies in the AFFINITY program were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki. In the surgical substudies, 28 patients underwent 35 procedures requiring general, spinal or regional anaesthesia. Dosing was guided by WFH recommendations. rVIII-SingleChain was used either as a bolus or continuous infusion. Haemostatic efficacy of rVIII-SingleChain during surgery was rated by investigators.

Results:

Surgical procedures performed were: abdominal hernia repair, ankle arthroplasty, ankle hardware removal, appendectomy, arthrodesis of the ankle joint, cholecystectomy, circumcision (9), debridement (2), elbow replacement, excision curettage and bone grafting, dental extraction (3), knee arthroscopy, knee replacement (7), knee spacer and immobilization, lengthening of achilles ligament (2), open reduction and internal fixation of ankle and port-a-cath removal. 27 surgeries were performed using bolus infusion while 8 surgeries used continuous infusion of rVIII-SingleChain. In 32 (91%) procedures efficacy was rated as excellent (defined as haemostasis not clinically significant different from normal) and in 3 (9%) surgeries efficacy was rated as good (defined as haemostasis normal or mildly abnormal in terms of quantity and/or quality eg, slight oozing). No related AEs or SAEs were observed during the surgery period.

Conclusion:

rVIII-SingleChain provides very effective and safe control of haemostasis during a wide range of surgical procedures when dosed either by bolus or continuous infusion.

453. Health-related quality of life in paediatric haemophilia B patients treated with rIX-FP

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Background:

Bleeds, frequent infusions and pain can impact the health-related quality of life (HRQoL) in children with haemophilia, limiting their ability to lead a normal life. In a multi-centre Phase III study (NCT01662531) paediatric patients received weekly prophylaxis with rIX-FP (IDELVION).

Aims:

To determine the impact of rIX-FP prophylaxis on HRQoL in paediatric patients.

Methods:

27 children (mean age 5.9±2.93 years) were enrolled. HRQoL was assessed at baseline and at the end of study (EOS) (≥12 months later). Children completed the haemophilia-specific HRQoL questionnaire (HAEMO-QOL) and their caregivers the haemophilia-specific treatment satisfaction (TS) questionnaire (HEMO-SATP). Scores range from 0–100; decreasing scores indicate improvements in HRQoL and TS. Minimal important difference (MID) between baseline and EOS were calculated based on the Cohen's *d* effect size (ES). ES of *d*=0.2 indicate small effects, *d*=0.5 medium, and *d*=0.8 large effect size.

Results:

At baseline 19 children (age group I: 4-7 years (n=12), age group II: 8-12 years (n=7)) completed the respective age group version of the Haemo-QoL, 23 parents filled in the Hemo-SatP. Children reported good HRQoL (I: M=19.07±16.9; II: M=26.09±6.3); parents showed a high TS in the Hemo-SatP (M=14.45±11.2). HRQoL differences between baseline and EOS were not statistically significant in age group I, but a medium ES was found for 'physical health' (*d*=.601) and 'treatment' (*d*=.491). Age group II showed a statistically significant improvement from baseline to EOS in the Haemo-QoL total score (*p*<.037), medium to big ES were found for most of the domains. Parents reported a statistically significant TS improvement in the dimension 'burden' of the Hemo-SatP (*p*<.034), small ES were found for most of the domains.

Conclusion:

Children showed HRQoL improvement between baseline and EOS, this difference reached statistical significance in age group II. Parents reported a significant improvement in TS.

454. Magnitude of dosing adjustment of AFSTYLA in clinical trials

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Background:

AFSTYLA (rVIII-SingleChain) is indicated in adults, adolescents and children with hemophilia A for control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and for perioperative management. In one adult/adolescent (12-65 years) trial and one pediatric trial (<12 years), dosing was according to investigator's discretion in order to reflect practice in the clinical setting. Adjustment of dose or frequency was allowed at any time during the studies.

Aim:

To estimate the difference in factor use of AFSTYLA between the final and the initial dosing regimens (DELTA), in two pivotal clinical trials.

Methods:

Factor use per week for prophylaxis was calculated based on frequency of injection and dose per injection, for the initial and final dosing regimens. DELTA was then taken (in IU/kg per week, for prophylaxis). Patients were grouped by initial dosing frequency and DELTA was summarized for each group (in which the same patients were followed).

Results:

In the adult/adolescent trial, 47 and 79 subjects initially received two and three injections per week, respectively. DELTA ≤ 0 occurred in 78.72% of subjects receiving 2 injections per week and 77.22% of subjects receiving 3 injections per week. In the pediatric trial, 43 subjects initially received 2 injections per week with DELTA ≤ 0 in 69.77% of the subjects; 24 subjects initially received 3 injections per week with a DELTA ≤ 0 in 75.00% of the subjects.

Conclusions:

The majority of subjects had the same or decreased factor use between the initial and final dosing regimens. Although real world factor consumption of AFSTYLA is yet to be measured, these results indicate that it is likely to be predictable and stable during the course of treatment.

455. Prophylaxis with rIX-FP reduces consumption compared with previous FIX in both adult and paediatric patients

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Background:

rIX-FP, a fusion protein genetically linking recombinant human coagulation Factor IX with recombinant human albumin, was designed with an improved pharmacokinetic profile to extend the dosing interval during prophylaxis therapy (PT).

Aims: To evaluate the impact of an extended dosing interval on FIX consumption in adult and paediatric patients with haemophilia B enrolled into 2 clinical trials.

Methods:

Study 1: Previously treated patients (12–65 years) with haemophilia B (FIX \leq 2%) received either on-demand treatment with rIX-FP for 6 months before switching to 7-day PT (n=23) or received 7-day PT for 6 months and continued on 7-day PT or switched to 10- or 14-day PT (n=40). Study 2: Paediatric patients (<12 years; n=27) received 7-day PT with rIX-FP. Consumption of FIX before and during study was compared.

Results:

In adults, 7- and 14-day PT with rIX-FP reduced mean monthly FIX consumption compared with previous FIX therapy by approximately 37% and 51%, respectively (7-day: 202.7 IU/kg; 14-day: 157.4 IU/kg; previous FIX: 320.7 IU/kg). Mean dose for patients receiving 7-day PT (n=59) was 47.1 IU/kg versus a mean weekly dose prior to study entry of 69.9 IU/kg. Mean dose for 14-day rIXFP PT (n=21) was 71.9 IU/kg. In the pediatric population, treatment frequency in those receiving PT prior to study entry was 2x weekly (n=15), 3x weekly (n=2), every 3 days (n=2), every other day (n=1) and weekly (n=4); 7-day rIX-FP PT decreased injection frequency for 20/24 patients. Mean weekly dose was considerably lower with rIX-FP PT than with previous FIX for all patients (47.2 vs 107.1 IU/kg), for those aged <6 years (49.1 vs 138.7 IU/kg) and for those 6 to <12 years (45.6 vs 80.3 IU/kg).

Conclusion:

Compared to previous treatment, prophylaxis with the prolonged dose interval afforded by rIX-FP resulted in a significant decrease in total FIX consumption and a subsequent reduction in the burden of treatment in this group of patients.

456. A Rare Case of a Factor V Inhibitor Detected in Association with Bee Sting Anaphylaxis

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Introduction:

Acquired factor V inhibitors are considered rare events with most historical cases being associated with the use of topical bovine thrombin, or antibiotic therapy in critically unwell patients¹.

Case:

A 25 year-old, previously well male, presented with anaphylactic shock following a honeybee sting. He was transferred to hospital after a period of prolonged hypoxia and hypotension. Upon arrival in the emergency department a definitive airway was established with endotracheal intubation. Computer tomography (CT) of the brain revealed changes consistent with hypoxic brain injury. The initial coagulation profile demonstrated critically prolonged prothrombin (PT), activated partial thromboplastin (APTT) and thrombin times (TT), which did not correct with a 50/50 mix. Additionally, there was marked hypofibrinoginaemia. The patient subsequently developed haematuria and haematochezia and was administered fresh frozen plasma and cryoprecipitate for the management of presumptive disseminated intravascular coagulopathy (DIC).

Factor levels performed on pre-transfused samples showed critically low levels of factors V, XIII, IX and XI, which did not correct following transfusion of plasma products. A factor V inhibitor was detected at 40 Bethesda units (at sample taken 6 hours after arrival at hospital). Unfortunately, the patient's clinical status declined and a repeat CT brain demonstrated progression of hypoxic brain injury with mass effect and cerebral tonsillar herniation. In the absence of ongoing plasma product administration, clotting times normalised 18 hours after presentation. The patient was subsequently pronounced dead and underwent organ retrieval.

Discussion:

The venom of the honeybee contains phospholipase A2, which in vitro has been shown to influence all phases of the coagulation cascade^{2,3}. Interestingly, in our case the prolonged clotting times appeared to be time dependent, thus leading to the postulations that exposure to honey bee venom induces a cross-reacting antibody to human FV or contains a FV-like inhibitor.

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457. Efficacy, safety and tolerability of a new intravenous immunoglobulin 10% in primary chronic ITP

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Aim:

This pivotal trial assessed the efficacy of a new human normal IVIG 10% solution in correcting the platelet count in patients with primary chronic ITP.

Methods:

In this prospective, open-label, multicentre, phase III study, patients received a daily dose of 1 g/kg IVIG 10% for 2 consecutive days. Twenty centres in Bulgaria, Czech Republic, Germany, India, Poland, Romania, Russia and Ukraine enrolled patients. Primary endpoint was the clinical response rate defined as increase in platelets to $\geq 50 \times 10^9/L$ within 7 days after first infusion; secondary endpoints included alternate response rate definitions, time to response, response duration, platelet counts, regression of bleeding, and safety. Analyses were performed on the full analysis set (FAS) consisting of all enrolled patients with at least one post-baseline platelet concentration measurement. Analyses were conducted using statistical software SAS (version 9.1 or higher).

Results:

Forty patients were enrolled (57.5% male, mean age 36.7 years, range 18–72); the FAS comprised 36 patients. Clinical response was seen for 29 of 36 patients (80.6%; mean maximum platelet count of $237 \times 10^9/L$); in 18 of 23 patients (78.3%) with bleedings at baseline, haemorrhages completely resolved by day 8, confirming the efficacy of the 10% IVIG. Median time to response and response duration was 2 days and 14 days, respectively. The new human normal IVIG 10% solution was well tolerated at a maximum infusion rate of 8 mg/kg/minute in all but one patient; adverse events were mainly mild to moderate in severity. The most frequent was headache (42.5% of patients) followed by pyrexia (22.5% of patients). The death of two patients was assessed as unrelated to study drug.

Conclusion:

The study drug was well tolerated even at high infusion speed and induced a rapid platelet count increase, thus decreasing the bleeding rate and severity of bleeding events.

458. Severe acquired Haemophilia A, in the setting of homozygous Factor V Leiden deficiency and thrombosis.

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Introduction:

Acquired haemophilia A (AHA) is a rare, life threatening bleeding disorder due to autoantibodies directed against factor VIII (FVIII). AHA can be seen in elderly patients and can be associated with conditions including malignancy and autoimmune disorders.

Method:

We describe a 73 year old lady on stable Warfarin therapy for 10 years for an unprovoked above knee deep vein thrombosis with a known inherited homozygous Factor V Leiden mutation. She had increasing bleeding since February, initially thought to be secondary to Warfarin despite a therapeutic INR. In May, she was admitted with severe haemoptysis, and had a markedly abnormal coagulation profile. She was incidentally noted to have serological evidence of past Hepatitis B infection.

Results:

Prothrombin time was 20 seconds (s) (normal 10-15s), corresponding to an International Normalized Ratio of 1.7 (normal 2-4.5), and activated partial thromboplastin time (aPTT) was 119s (normal 25-36s). The disproportionately prolonged aPTT led to further investigations confirming an acquired factor VIII inhibitor. FVIII activity was undetectable (normal 50-150s) and the inhibitor was greater than 1000 Bethesda unit (BU)/ml at dilution level 640.

The patient was treated with high dose steroids and weekly rituximab. Rituximab is an anti-CD20 monoclonal antibody shown to be successful in inhibitor eradication. After four doses of rituximab, the inhibitor level reduced to 84BU/ml and the aPTT to 50s. Ongoing treatment and monitoring for Hepatitis B reactivation continues.

Conclusion:

Severe AHA in the setting of known inherited thrombophilia and anticoagulation with successful early rapid response to high dose steroids and Rituximab therapy. This case highlights the need to consider alternative diagnostic possibilities in patients with an atypical presentation as well as the difficult balance between strong thrombotic and bleeding tendencies.

459. Evaluation of Anthocyanins effects to alleviating platelet activity

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Background:

Platelet hyperactivity has a significant role in initiating vascular thrombosis and subsequent cardiovascular disease (CVD). Many studies demonstrated resistance to currently used antiplatelet therapies such as aspirin. Antioxidant activity of polyphenolic compounds has been shown to reduce platelet activity.

Aim:

Assessment of the *in vivo* effect of pure AC compounds on the platelet and aggregation and activation.

Methods:

Healthy human subject (n=26) were recruited in this study. Each participant has consumed 80 mg of AC/day in the form of Medox[®] capsules for 28 days. Fasting blood sample was collected at baseline and after the treatment period. Flow cytometry was used to assess platelet activation by measuring platelet surface marker expression of CD41a and P-selectin in response to adenosine diphosphate (ADP).

Results:

Flow cytometric analysis showed a significant suppressive effect of AC at a dose of 320 mg/day on the expression of P-selectin as measured by the platelet surface expression of CD62p.

Conclusion:

The results show that AC may reduce platelet activation as demonstrated by inhibition of P-selectin expression. These results provide greater insight into the effect of AC and the possible mechanism by which AC can reduce platelet activation. Hence, AC may act as a compliment to other anti-platelet agents to reduce the occurrence of thrombotic events.

460. Underestimation of anticoagulation with unfractionated heparin when using reduced volume collection tubes

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Aims:

Reduced volume (RV) collection tubes may be used to limit iatrogenic blood loss or in cases of difficult venipuncture. The published literature suggests that while adequate for standard coagulation assays and monitoring patients on warfarin or low molecular weight heparins, RV tubes may be unreliable for unfractionated heparin (UFH) monitoring. Heparin neutralization is thought to occur due to increased levels of platelet factor 4 as a result of increased platelet activation in the larger air space present in these tubes.[1, 2] To ensure patient safety is maintained when using RV tubes, a local study was conducted comparing APTT and anti-Xa results between RV and standard volume (SV) tubes in samples taken from patients on UFH.

Method:

Twenty-two samples were obtained at a frequency consistent with normal practice. On each occasion 7.5mL of blood was withdrawn to fill one 3.5mL (SV) and two 2mL (RV) tubes. The SV tube and one of the RV tubes were centrifuged within one hour; the second RV tube was centrifuged after 2-3 hours to obtain data regarding the effect of timely centrifugation on the results. Mean APTT and anti-Xa levels were compared.

Results:

SV versus RV tube processed within one hour: mean APTT (sec) 91.4 versus 76.8, difference 14.6 (8.8-20.3); mean anti-Xa (IU/mL) 0.53 versus 0.43, difference 0.10 (0.06-0.13); $p < 0.00005$ for both.

RV tube processed within one hour versus RV tube processed after 2-3 hours: mean APTT (sec) 76.8 versus 67.9, difference 8.9 (4.7-13.0), $p < 0.0005$; mean anti-Xa (IU/mL) 0.43 versus 0.36, difference 0.07 (0.05-0.10), $p < 0.00005$.

Conclusion:

RV collection tubes significantly underestimate the degree of anticoagulation with UFH and should not be used in monitoring for this purpose. Secondly, our study reconfirms the importance of pre-analytical variables- such as timely transport and processing of samples- in maintaining accurate results and patient safety when monitoring anticoagulant therapy.

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461. Stability of Rivaroxaban and Apixaban levels by Anti Xa assay in blood samples.

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Direct Oral Anticoagulants (DOACs) are being prescribed more commonly as anticoagulant therapy in place of warfarin. In some circumstances a patient may have blood collected for coagulation studies without the requestor knowing that they take a DOAC (e.g. in the emergency department) and then much later a drug assay is requested on the initial sample. The aim of our study was to determine if the concentration of two common DOACs, Rivaroxaban (RIVA) and Apixaban (APIX), could be accurately measured at times that exceed recommended processing time limits. This would allow for the requesting of the drug levels >6hrs after the sample was collected.

Rivaroxaban and Apixaban were measured using a Stago Sta-R analyser and the Stago STA Liquid Anti Xa kit with corresponding calibrators and quality control material. Twenty RIVA and 38 APIX citrated blood samples were centrifuged, tested and reported within 6 hours of collection, according to the laboratory's procedures. The residual spun plasma was left in the original blood tube at 2-4°C for 18-24 hrs post collection time and re-analysed. There was no significant change in results comparing analysis of RIVA or APIX at less than 6 hours (mean time post collection = 2hrs 30mins) to greater than 18 hours from collection. (RIVA range 12-565 U/mL, mean difference -2.75 U/mL, p=0.44 and r²= 0.99. APIX range 12-400 U/mL, mean difference -2.88 U/mL, p=0.093, r²= 0.99).

In conclusion, Rivaroxaban and Apixaban can be measured in centrifuged plasma that has been stored for up to 18 hours at 2-4°C post collection. This allows testing to be requested at a later time if a patient's medication history is updated to include that they take a DOAC and a blood level is required for management.

462. Venous thromboembolism prophylaxis of the obese obstetric patient – a cross-sectional study and literature review

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Aim:

Obesity increases the risk of venous thromboembolism (VTE) in obstetric patients regardless of delivery mode and for up to 6 weeks postpartum. This study aimed to evaluate postpartum pharmacological VTE prophylaxis practices for obese patients at an Australian metropolitan tertiary hospital, and to review available literature.

Method:

Medical records of obstetric patients with BMI \geq 30 who delivered during March 2016 – March 2017 were examined for demographic data, VTE risk factors, and VTE prophylaxis. A review of literature describing postpartum VTE prophylaxis in obesity was also undertaken.

Results:

Fifty-seven postpartum patients (36 caesarean section cases and 21 vaginal births) were reviewed. All caesarean section patients were considered to have \geq 1 inherent VTE risk factor in addition to obesity, and 15 vaginal delivery patients had \geq 2 additional risk factors. Thirty-seven of 57 (65%) patients had low molecular weight heparin (LMWH), dalteparin or enoxaparin, prescribed during their inpatient stay. Of the 36 caesarean section patients and one vaginal delivery patient (BMI \geq 40 with \geq 2 risk factors) who received LMWH, 15 (41%) received the recommended weight-adjusted dose. Two patients, one declining, were offered extended-course LMWH on discharge.

Studies examining VTE prophylaxis in obese obstetric patients are limited. The majority of literature recommends prophylactic LMWH for BMI \geq 30 +/- additional risk factors, and acknowledges the inadequacy of fixed-dose LMWH. Some discrepancies in indication criteria exist between guidelines and dose-adjustment details are not consistently specified. Dosing of 60-80mg/day for enoxaparin and 7500-10000units/day for dalteparin has been recommended, with durations ranging from 1-6 weeks.

Conclusion:

Use of pharmacological VTE prophylaxis in obese postpartum patients and prescribing compliance for weight-adjusted and extended courses of LMWH appears variable. Limited literature, recommendation discrepancies, and varied awareness of recommendations may be contributing factors. Further education and research is warranted regarding this high-risk patient group.

*No conflict of interest to disclose.

463. The unusual case of JV

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This poster documents the unusual presentation of JV, a 17 year old female who attended Westmead Hospital in 2013 with pain and extensive atraumatic bruising covering her buttocks, anterolateral thighs and calves. She reported experiencing 4 weeks of myalgias, for which she had been taking over the counter analgesics. Her bruises were tender to touch, but no pain elsewhere.

Extensive medical consultation occurred and JV was tested for an autoimmune cause to the problem. Returned blood results demonstrated an abnormal haematological profile with a profound hypofibrinoginaemia (<0.6g/L). This was coupled with unrecordably high DsDNA antibodies, ANA and ENA, and low Complement C3 & C4, consistent with diagnosis of systemic lupus erythematosus (SLE).

Acquired hypofibrinoginaemia is not reported extensively in the literature. While there are some case reports, these are mainly the result of depletion of fibrinogen levels due to bleeding, therapy or drug-induced hypofibrinoginaemia, and those secondary to haematologic disorders such as Multiple Myeloma.

Whilst undergoing testing and treatment, JV required multiple transfusions of plasma products to maintain a functional fibrinogen level, necessitating 1:1 care in a specialist haematology ward with nurses and medical staff familiar with the needs of bleeding patients and frequent delivery of blood products. Transfusion laboratory staff were intimately involved in the sourcing and monitoring of JV's transfusion requirements, and were able to establish and maintain access to Fibrinogen concentrate – ultimately using the entire state supply and requiring urgent dispatch from interstate to meet the hospital's demand. Westmead's specialist coagulation scientist was at an international conference and was able to discuss the case with leading experts and communicate feedback in real time to the treating teams, allowing for prompt diagnosis and decision making.

This case, whilst being interesting and unusual, also illustrates how effective team work between multiple disciplines can ensure positive patient outcomes and survival.

464. Thrombin generation as assessed using the Ceveron® Alpha analyser as a measure of apixaban and rivaroxaban pharmacodynamics

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Aim:

Thrombin generation assays (TGA) are global coagulation assays which may correlate more closely to a patient's haemostatic profile compared to conventional clotting-time assays, including those on therapeutic anticoagulation. Plasma concentrations of direct FXa inhibitors (e.g. apixaban, rivaroxaban) can be quantified using calibrated anti-FXa assays; however, these assays do not provide information about the pharmacodynamic effects of the anticoagulants. Our aim was to establish whether the Ceveron® Alpha analyser TGA (Technoclone, Austria) is a reliable measure of direct FXa inhibitor pharmacodynamics.

Method:

Blood samples from normal controls (n=17), and patients treated with apixaban (n=13) or rivaroxaban (n=12) were collected. All patients included in this analysis had FXa inhibitor plasma concentrations (>30ng/mL) as confirmed using calibrated anti-FXa assays. Samples were double spun and frozen (-80°C) until testing. Tissue factor (3-5pM) was used to initiate coagulation. TGA parameters assessed included the AUC, lag time, peak height, time to peak (tPeak) and velocity index (VI).

Results:

The median (range) concentration for apixaban and rivaroxaban treated patients were 75ng/mL (44 – 335ng/mL) and 67ng/mL (32 – 234ng/mL), respectively. The median (range) TGA parameters for each group are shown below:

	Lag (min)	AUC (nM ² min)	tPeak (min)	Peak (nM)	VI (nM/min)
Control	4.1 (3.3 - 4.9)	1618 (1258 – 2059)	10.9 (8.9 – 12.7)	102 (81 - 176)	14.4 (10.7 – 33.1)
Apixaban	4.5 (2.9 - 7.1)	1149 (292 - 1543)	21.6 (9.6 - 27.4)	36 (10 - 55)	2.2 (0.5 - 9.7)
Rivaroxaban	5.3 (4.3 - 7.3)	1106 580 - 2486	20.3 (15.7 - 36.5)	36 (19 - 107)	2.4 (0.9 - 8.1)

Conclusion:

The Ceveron® Alpha TGA analyser can quantify the pharmacodynamic effects of direct FXa inhibitor anticoagulants. Patients treated with apixaban or rivaroxaban demonstrated marked inhibition of thrombin generation compared to normal controls.

465. A comparative analysis of thrombin generation assay analysers

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Aim:

Thrombin generation (TG) is a global coagulation assay which better describes an individual's haemostatic profile compared to standard clotting-time based coagulation tests. The Calibrated Automated Thrombogram (CAT; Stago, France) and the Ceveron® Alpha analyser (Technoclone, Austria) both measure TG, with the CAT well established in Australia. However, it is uncertain how the 2 platforms compare. Our aim was to establish a normal range (NR) for the Ceveron® Alpha (Ceveron) and CAT analysers, and to subsequently validate and compare TG parameters across platforms.

Method:

17 citrated samples from healthy donors and 30 plasma standards (Siemens) were used to establish a NR per manufacturer's recommendations, using the Ceveron and the CAT, respectively. A subsequent pilot validation cohort of healthy donor plasmas (n=9) was compared using both platforms. Collected samples were double spun and frozen (-80°C) until testing. Both platforms used equal amounts of tissue factor (3-5pM) to initiate coagulation. TG parameters assessed included the AUC, lag time, peak height, time to peak (tPeak) and velocity index (VI).

Results:

	Lag (min)	AUC (nM [*] min)	tPeak (min)	Peak (nM)	VI (nM/min)
<i>Normal Range (NR) derived for each analyser (independent samples):</i>					
CAT	2.1 – 4.1	1500 – 2835	3.8 – 7.8	160 – 320	26 – 86
Ceveron	3.2 – 5.0	1164 – 2111	9.0 – 12.8	56 – 171	4 – 30
<i>Observed range (min - max) within validation cohort (same samples):</i>					
CAT (% within NR)	2.0 – 4.1 (88.9%)	528 – 1544 (11.1%)	4.0 – 8.9 (77.8%)	64 – 302 (44.4%)	16 – 151 (55.6%)
Ceveron (% within NR)	1.4 – 4.4 (77.8%)	1099 – 2280 (66.7%)	3.8 – 13.0 (66.7%)	64 – 321 (77.8%)	8 – 133 (77.8%)

Conclusion:

Our results demonstrate platform-dependent TG values, highlighting the need for laboratories to determine their own NR for their analysers. Despite broad similarities in TG parameters, there remains discrepancies between the platforms and lower than expected performance within the pilot validation cohort. Continued validation of these comparative results using an expanded cohort will be presented.

466. Relationships between thrombin generation, procoagulant microparticles, in-vitro thrombotic tendency and clinical thromboembolism in acquired coagulopathy.

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Aims: Identifying which patients with deranged coagulation parameters or acquired coagulopathy are at risk of subsequent thromboembolism, and hence may potentially benefit from early anticoagulant prophylaxis, is difficult. This pilot study aimed to assess whether plasma thrombin generation and procoagulant microparticle concentrations were useful to identify patients who were at risk of increased in vitro thrombotic tendency or subsequent clinical thromboembolism.

Methods: Forty critically ill patients who had at least one deranged coagulation parameter (INR >1.5, aPTT >40s, or platelets <150x10⁹/L) were recruited into this prospective cohort study. The relationships between plasma thrombin generation (measured by Calibrated Automated Thrombogram), thrombin-antithrombin complexes (Siemens Enzygnost[®] TAT), prothrombin fragments (Siemens Enzygnost[®] PF1.2), procoagulant microparticles concentrations (XACT[®] test for detection of procoagulant plasma phospholipid) and risk of increased in vitro whole-blood thrombotic tendency or subsequent clinical thromboembolic events were assessed. A maximum amplitude on the thromboelastography (TEG[®]) >72mm □ measured simultaneously with the other coagulation assays □ was defined as increased in vitro thrombotic tendency in this study.

Results: Of the 40 patients included in the study, nine (22.5%) and eight patients (20%) had increased in vitro thrombotic tendency and subsequent clinical thromboembolic events, respectively. Thrombin generation (median 1044 vs 1148nM*min, p=0.377), prothrombin fragments (455 vs 448pmol/L, p=0.377), thrombin-antithrombin complexes (17.5 vs 7.7ug/L, p=0.082), and procoagulant microparticles (36.8 vs 34.5ug/mL, p=0.454) were not significantly different between patients with and without subsequent thromboembolic events. Similarly, thrombin generation (p=0.726), prothrombin fragments (p=0.321), thrombin-antithrombin complexes (p=0.633), and procoagulant microparticles (p=0.388) were also not different between patients with and without increased in vitro thrombotic tendency.

Conclusion: This pilot study could not confirm the ability of using thrombin generation, thrombin-antithrombin complexes, prothrombin fragments and microparticle concentrations to predict either increased in vitro thrombotic tendency or risk of subsequent clinical thromboembolic events in critically ill patients with acquired coagulopathy.

467. Viscoelastic testing to predict subsequent clinical thromboembolic events: A systematic review and meta-analysis

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Aims: There is a clear need to individualise thromboembolic prophylaxis by selecting patients who are most likely to benefit, while avoiding the unnecessary risks for those at low-risk of thromboembolism. This systematic review and meta-analysis critically analysed whether a hypercoagulability, identified by viscoelastic point-of-care (VE-POC) test, is predictive of subsequent clinical thromboembolic events.

Methods: We searched for relevant studies in the Cochrane, MEDLINE and EMBASE databases to the 1st of March 2017, using the following free-text and exploded Medical Subject Headings: “thrombosis”, “venous thrombosis”, “venous thromboembolism”, “deep vein thrombosis”, “pulmonary embolism”, “prothrombotic”, or “thrombotic” with “viscoelastic point-of-care”, “thromboelastography”, “thromboelastometry”, “rotational thromboelastometry”, “TEG”, or “ROTEM”. This review has been registered with PROSPERO: CRD42017057968.

Results: Data from 38 studies involving 8748 patients, suggested a hypercoagulability, identified by a VE-POC test, had a moderate ability to predict thromboembolism (area under the summary receiver-operating-characteristic [sROC] curve=0.705, 95% confidence interval [CI]:0.651-0.759). The pooled sensitivity, specificity, and diagnostic odds ratio (DOR) to predict thromboembolic events were 0.549 (95%CI:0.419-0.674), 0.776 (95%CI:0.688-0.845), and 3.699 (95%CI:2.608-5.247), respectively. The predictive performance of the VE-POC test varied slightly between different patient populations (cancer: sROC=0.773, trauma: sROC=0.659, perioperative: sROC=0.691, critically ill: sROC=0.769). Compared to TEG[®], ROTEM[®] had a better ability to predict thromboembolism (sROC and pooled DOR 0.686 and 3.263 versus 0.778 and 6.268, respectively). Publication bias was not observed.

Conclusion: These results suggest that patients with a hypercoagulable VE-POC result are associated with a higher risk of clinical thromboembolic events, and this may be helpful to assist the decision to initiate anticoagulant prophylaxis. A normal VE-POC result is less helpful clinically. Careful consideration of other clinical and laboratory factors is needed to balance the benefits of anticoagulant prophylaxis and its potential harms on increased risk of bleeding.

468. Synergistic interplay of an ACE910 sequence identical analog and a bypassing reagent and its components

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Aims:

Investigational non-factor products such as ACE910, an antibody to FIX(a) and FX(a), may offer new treatment options for hemophilia patients with inhibitors; however, its unregulated mechanism of action raises questions regarding clinical safety and efficacy. We determined the source of excessive in-vitro coagulation with a sequence identical analog of ACE910 (SIA) combined with bypassing agent FEIBA.

Methods:

SIA was analyzed in thrombin generation (TG) experiments using platelet poor plasma (PPP) from hemophilia A inhibitor patients and hemophilia A plasma reconstituted with platelets from 16 healthy donors (PRP). A normal TG range was established in healthy donor plasma. Therapeutic doses of SIA (20-600 nM) were tested alone and with FEIBA (0.05-1 U/mL) or rFVIIa (0.88-5.25 µg/mL). To measure FEIBA components' contribution to the synergistic effect with SIA, PPP was spiked with purified plasma proteins. Clot formation was analyzed in FVIII-inhibited blood by ROTEM and T-TAS.

Results:

Normal peak thrombin range was 47-144 nM (PPP) and 88-231 nM (PRP). rFVIIa and FEIBA had a synergistic effect on TG in combination with SIA in PPP and PRP. Combined with rFVIIa (0.88 µg/mL) or FEIBA (0.5 U/mL), SIA (600 nM) induced ~2- and ~16-fold increases over SIA alone. SIA+rFVIIa did not reach the normal range, while SIA+FEIBA far exceeded it. Clot formation in FVIII-inhibited whole blood confirmed the synergistic effect of SIA+FEIBA. Adding individual FEIBA components to PPP showed that FIX was, with a half-maximal effect, the main driver for enhanced TG, followed by FIXa.

Conclusion:

Excessive thrombin generation and faster clot formation occurred when combining SIA at presumed clinical concentrations (ACE910 study NCT02622321) with FEIBA. In vitro, this effect is mainly mediated by FEIBA component FIX. ACE910 binds to FIX and FIXa, and displays its pro-coagulant effect via an unregulated mechanism. Therefore, careful judgment is required in treating breakthrough bleeds with FEIBA.

469. A bispecific antibody lacks measurability in routine coagulation assays and comparability to factor VIII

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Aim:

For diagnostic management of hemophilia A patients receiving ACE910 as alternative treatment, e.g. FVIII chromogenic assay, activated partial thromboplastin time (APTT), and thrombin generation assay (TGA) are under evaluation. This study examined the effect of assay type on hemostatic activity monitoring of a sequence identical analogue to ACE910 (SIA) and assessed FVIII activity equivalency.

Methods:

SIA was analyzed in 4 commercial chromogenic assay kits; rFVIII served as comparator. Clotting times were measured by APTT using 5 differently composed trigger reagents followed by clot waveform analysis. In TGA, SIA was tested using extrinsic and intrinsic trigger conditions. As needed, concentrations ranged from 2–1200 nM.

Results:

Lack of cross-reactivity of SIA to bovine factors rendered only the Biophen FVIII:C test suitable for analysis. As the dose response of SIA and FVIII was not collateral, the chromogenic assay is not a reliable test for FVIII equivalence assessment. APTT was highly sensitive to SIA, which resulted in substantial reduction of clotting time at low and potentially non-therapeutic concentrations. Different APTT reagents yielded a FVIII equivalence range of 18%–69% (20 nM SIA) and >200% at a presumed clinical concentration of 600 nM. SIA (600 nM) only partially restored TG in hemophilia A patient plasmas, resulting in FVIII equivalents of 4%–8% (intrinsic) and 16%–36% (extrinsic) based on peak thrombin. Assessment of other TG parameters resulted in FVIII equivalency ranging from 0% to >100%.

Conclusions:

Analysis of SIA and derivation of FVIII equivalence therefrom is challenged by its novel mechanism of action/regulation. FVIII equivalence of SIA cannot be determined using standard FVIII protocols and is highly influenced by assay type, analytical conditions, and parameters used for calculation. Thus, new methods and a product specific standard are required for better prediction of the hemostatic effect of non-factor therapies.

470. P-selectin as a marker of cardiovascular risk in normal controls and myeloproliferative neoplasm

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Aim:

P-selectin is an adhesion molecule secreted by both the endothelium and platelets and has been shown to be higher in patients with coronary artery disease and those with cardiovascular risk factors. Given its role in inflammation and thrombogenesis, we evaluated its role in normal controls as well as those with myeloproliferative neoplasm (MPN).

Method:

Normal controls, without history of cardiovascular or thrombotic disease, and patients with MPN were recruited. Thromboelastography was performed on whole blood while the remaining global coagulation assays (calibrated automated thrombogram, overall haemostatic potential and P-selectin) were performed on thawed double-spun platelet-poor plasma stored at -80°C.

Result:

Eighty-nine normal controls (59 females, 30 males) and 37 MPN patients (20 females, 17 males) were recruited. MPN patients had markedly higher median P-selectin levels (109.9 vs 49.3 ng/mL, $p < 0.01$), and those with higher P-selectin levels had higher platelet counts ($p < 0.01$). Higher P-selectin levels were also associated with lower vWF activity (74% vs 101%, $p = 0.04$) and factor VIII levels (91% vs 123%, $p = 0.02$). No differences were seen between P-selectin levels and the other global coagulation assays.

In normal controls, higher P-selectin is associated with older age, raised LDL (3.2 vs 2.8 mmol/L, $p = 0.03$) and triglycerides (1.5 vs 1.1 mmol/L, $p = 0.02$), as well as higher maximum amplitude (60.0 vs 56.0 mm, $p = 0.01$), and OHP (31.5 vs 26.9, $p < 0.01$). Interestingly, CAT parameters were lower with reduced velocity index (63.9 vs 83.0 nM.min, $p = 0.01$) and thrombin peak (206.2 vs 241.0 nM, $p = 0.02$).

Conclusion:

P-selectin is markedly higher in MPN patients and is likely related to increased platelet secretion, however, interestingly this did not impact global coagulation assays and is associated with decreased factor VIII and vWF levels. In normal controls, higher P-selectin is associated with poor lipid profile, older age and male sex, and could serve as a marker of cardiovascular risk.

471. Effects of anti-beta 2 glycoprotein 1 antibodies on collagen-induced platelet aggregation

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Aim:

Anti-beta-2-glycoprotein 1 (anti- β_2 GP1) antibodies are associated with increased thrombotic risk in autoimmune diseases patients. There is conflicting evidence on the effects of anti- β_2 GP1 antibodies on platelets, with both enhanced and inhibited aggregation previously reported. The aims of this study were to 1) investigate the effects of different anti- β_2 GP1 antibodies on collagen-induced platelet aggregation in parallel with negative/baseline and isotype control, and 2) determine lupus anticoagulation (LA) activity of anti- β_2 GP1 antibodies used.

Method:

Three animal-derived anti-human- β_2 GP1 or isotype control antibodies (final concentrations 1.25, 2.5, 5 μ g/mL) were incubated with healthy platelet rich plasma prior to activation by collagen (2.5 μ g/mL). Parameters including maximum aggregation, area under curve and lag time were measured using light transmission aggregometer. Every concentration of antibody (triplicate) with a baseline control were tested in each run and repeated using plasma from four different healthy donors. Data were analysed using mixed effects general linear modelling adjusted for repeated measures. Anti- β_2 GP1 antibodies' effects were first compared against baseline. If statistically significant, results were compared to their isotype controls to ensure those effects were specific for anti- β_2 GP1 antibodies.

LA activity was measured by incubating normal plasma with anti- β_2 GP1 antibody. Screening reagent containing Russell's viper venom and phospholipids was added to plasma spiked with antibodies. Clotting times were measured using coagulation analyser in triplicate.

Result:

Each anti- β_2 GP1 antibody demonstrated some inhibition of aggregation compared to baseline control, but not to isotype control. Furthermore, no anti- β_2 GP1 antibody demonstrated LA activity, suggesting that antibodies used were probably non-pathological type B anti- β_2 GP1 antibodies.

Conclusion:

This study highlights both negative and isotype control are important to validate the effects of anti- β_2 GP1 antibodies on platelets. It is recommended to use assays that specifically measure anti-domain 1- β_2 GP1 antibodies, in conjunction with other functional measures e.g., LA activity, to investigate the effects of anti- β_2 GP1 antibodies on platelets.

472. Nonclinical assessment of FEIBA at a reduced volume reconstitution and higher infusion rate

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Aim:

To support the FEIBA STAR clinical study assessing different infusion rates and low volume reconstitution (ie, FEIBA reconstituted with 50% of the currently licensed amount of water for injection [FBA]), five nonclinical studies were conducted.

Methods:

Acute toxicity was evaluated in rats and FVIII-inhibited rabbits administered 100 U/kg FEIBA twice 6 hours apart at infusion rates of 2-100 U/kg/min. Efficacy of a reduced reconstitution volume was assessed in a FVIII-inhibitor nail clip model; rabbits were first treated intravenously (IV) with heat-inactivated FVIII-inhibitor goat plasma 45 minutes before nail cut and blood collection, then IV injected with FBA or FEIBA at 10, 50, or 100 U/kg body weight (BW) and blood collected for another 30 minutes. Endpoints included blood loss before/after treatment. Normal rabbits received FBA or FEIBA at 4 U/kg BW (infusion rate, 16 U/kg/min), and thrombus formation in the isolated jugular vein was scored. Local tolerance was evaluated after IV, intra-arterial, or paravenous injection into rabbits' ears.

Results:

In rats and rabbits, no correlation was observed between infusion rate and thrombogenicity. In rabbits, FBA treatment resulted in dose-related lower relative blood loss (71%, 2%, and 4% vs 104%, 3%, and 3% for FEIBA), statistically significant at 50 and 100 U/kg. Thrombus scores (FBA, ≤ 0.5 ; FEIBA, ≤ 2) demonstrated that FBA is no more thrombogenic than FEIBA at 1.6-fold the maximum infusion rate planned for clinical evaluation. FBA was well tolerated after IV and intra-arterial injection; paravenous injection caused mild local irritation.

Conclusion:

Results indicate comparable efficacy and safety for FEIBA and FBA. Infusion rates up to 100 U/kg/min (10-fold the planned clinical infusion rate) of the low reconstitution product revealed no adverse clinical signs. Thus, neither faster infusion rate nor lower reconstitution volume of FEIBA should pose additional risks to human subjects tested in a clinical study.

473. Laboratory monitoring issues in recombinant porcine FVIII replacement for a patient with acquired haemophilia A

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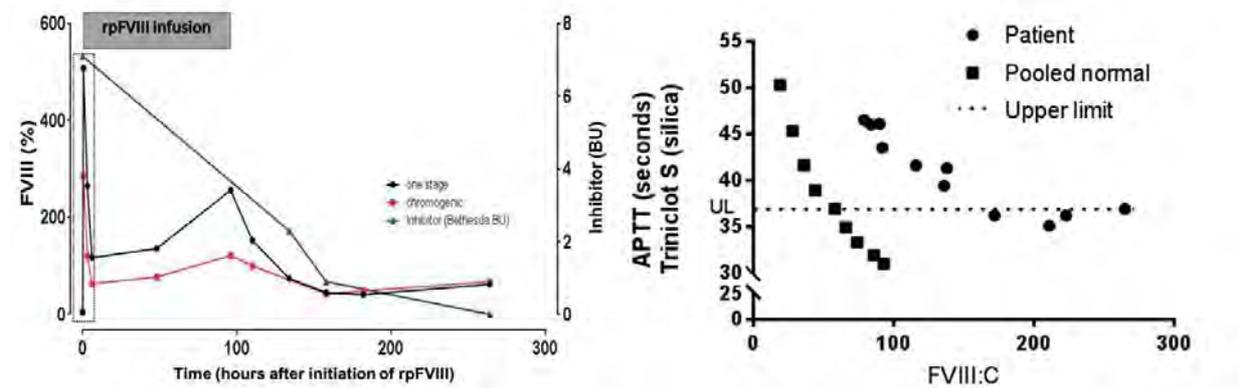
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Recombinant porcine sequence FVIII B-domain-deleted product (rpFVIII) is approved as replacement therapy for autoimmune haemophilia A (AHA). Here we describe a case of AHA associated with Bullous pemphigoid treated with rpFVIII whose course was complicated by a persistent non-correcting prolonged APTT and discrepancy between one-stage and chromogenic FVIII assays.

Aim: To highlight potential issues associated with laboratory monitoring of rpFVIII.

Methods: Patient plasma during rpFVIII infusion assayed by one stage FVIII clotting assay (Triniclot S, Stago STA-R), chromogenic FVIII assays (Hyphen kit, STA-R), 4 silica and 4 ellagic acid APTT reagents.

Results: In plasma from this patient with AHA, supra-therapeutic levels of FVIII were demonstrated after administration of the standard 200IU/kg loading dose of rpFVIII (508% by one stage assay and 279% by chromogenic). The APTT (Triniclot S) remained prolonged despite high levels FVIII measurable by Triniclot S based one-stage FVIII assay and APTT correction could not be used to direct rpFVIII infusion. Lupus anticoagulant and anti-porcine FVIII antibodies were excluded as causes. Although steady rpFVIII levels were achieved over first 48 hours, subsequent accumulation indicated a prolonged half-life in this individual. Chromogenic FVIII assays (Hyphen kit) on the STA-R analyser performed in parallel with one stage clotting assay on our patient showed a consistent significant discrepancy (ratio = 0.54, 0.47-0.65) until rpFVIII was cleared from plasma. Comparison of ICA (Rosner index) of 8 different APTT reagent mix tests with a uniform cut-off of 12.5% showed variable patterns of correction/non-correction not correlated with type of activator.



Conclusion: This is the first report of a non-correcting APTT after rpFVIII infusion. This was not related to type of activator and complicated monitoring of rpFVIII infusion rates in this individual. Chromogenic and one-stage clotting FVIII assays showed a consistent discrepancy, however, the current recommendation is use of clotting based assays for monitoring rpFVIII.

474. Results from guardian™2: using NovoEight® for prophylaxis and treatment in patients with severe haemophilia A

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Aim:

Turoctocog alfa (NovoEight®), a recombinant factor VIII (rFVIII) product, has been approved by major regulatory authorities for the prophylaxis and treatment of bleeds in patients with haemophilia A. guardian™2 was a large, multinational, extension trial of turoctocog alfa in paediatric and adult haemophilia A patients from 19 countries who were previously enrolled in the pivotal guardian™1 and guardian™3 trials, or who had completed pharmacokinetic trials. The aim was to assess the long-term safety and efficacy of turoctocog alfa for the prevention and treatment of bleeds.

Methods:

Previously treated patients, aged 1–70 years, with severe haemophilia A (FVIII activity $\leq 1\%$) without inhibitors, received prophylactic therapy with turoctocog alfa (20–50 IU/kg once every second day, 20–60 IU/kg three times weekly, or 40–60 IU/kg once every third day or twice weekly), with additional treatment for bleeding episodes. Treatment success was defined as a patient-reported 'excellent' or 'good' haemostatic response.

Results:

Data are included for more than 6 years from when the first patient was enrolled and a total of 753 patient-years of turoctocog alfa treatment (mean total number of doses: 164 per patient year for patients on prophylaxis; 55 per patient year for patients treated on-demand). No FVIII inhibitors (≥ 0.6 Bethesda Units) were detected and no safety issues were identified. The overall estimated annualised bleeding rate (ABR) (Poisson estimated mean) for patients on prophylaxis was 2.44 and the median ABR was 1.37 (Poisson estimated mean ABR for spontaneous bleeds was 1.34). The overall success rate for treatment of bleeds during the preventative regimen was 89.8%; 88.2% of all bleeds were successfully treated with 1–2 injections of turoctocog alfa.

Conclusion:

The end-of-trial results of guardian™2 show data that support the long-term safety and efficacy of turoctocog alfa in the prophylaxis and treatment of bleeds in patients with severe haemophilia A.

475. Early treatment benefit with room temperature-stable recombinant factor VIIa in haemophilia A/B with inhibitors (SMART-7™)

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Aim:

SMART-7™ investigated the safety/effectiveness of room temperature-stable rFVIIa (NovoSeven®) in patients with haemophilia A/B with inhibitors in a real-world setting. A subgroup analysis of the haemostatic response with rFVIIa treatment is presented.

Methods:

Study medication was not provided. Patient diaries were used to record bleeding episode information. Patients evaluated bleed status after treatment as 'bleed stopped', 'bleed slowed', or 'no change/worsened'. Overall bleed outcome was defined as the last given patient evaluation.

Results:

51 patients (1.6–69.5 years old; historical median bleeding rate: 1.0 episode/month) were enrolled; 31 (60.8%) completed the study; 20 discontinued. 48 patients experienced 618 bleeding episodes (median number/patient: 10.5; median study duration/patient: 13.9 months). 63.4% of bleeding episodes were spontaneous, and 31.2% were traumatic.

Effectiveness evaluation at end of treatment was available for 609 bleeding episodes: 93.4% resolved, 5.7% slowed and 0.8% were unchanged/worsened. Nine bleeds had no reported outcome. A total of 538 bleeds were treated with rFVIIa monotherapy: 94.2% resolved, 5.0% slowed and 0.7% were unchanged/worsened.

In a post-hoc analysis in which the data were divided by time to first treatment, the best haemostatic response (96.5%) was observed for treatment ≤1 h after bleed onset; effectiveness was also high (93.1%) for bleeds treated >1–≤4 h, decreasing for those treated >4 h after onset (87.3%; representing 13.1% of bleeds). Early treatment (≤1 h) with rFVIIa monotherapy was effective for both joint and muscle bleeds (96.2% and 97.3%, respectively). The initial rFVIIa dose administered was comparable for bleeds treated ≤1 h and those treated >1–≤4 h or >4 h after onset (median: 200.0, 197.2 and 202.1 □g/kg, respectively).

Conclusion:

High effectiveness with early rFVIIa treatment was demonstrated, with 96.5% of bleeds resolving when treatment was initiated ≤1 h after bleed onset; effectiveness remained high (93.1%) for bleeding treatment >1–≤4 h.

478. Global coagulation assays in normal control: the impact of age, ethnicity and gender

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Aim: There is no validated laboratory testing to evaluate an individual's complete coagulation profile. Global coagulation assays may be better surrogate measures of an individual's thrombosis risk. However, it is important to evaluate the differences within the normal population prior to the inclusion of these assays into studies on diseased populations.

Method: Normal controls with no thrombotic/cardiovascular history and no anticoagulant/antiplatelet use were recruited and evaluated with baseline laboratory investigations. Thromboelastography using citrated whole blood was performed with TEG 5000S and platelet-poor plasma were obtained for assessment of thrombin generation with calibrated automated thrombogram (CAT) and fibrin-aggregation (with and without tPA) with overall haemostatic potential (OHP) assay.

Result: Ninety-six normal controls with median age 44 years were recruited. Females had more prothrombotic TEG and CAT parameters ($p < 0.01$) as well as increased fibrin generation (overall coagulation potential, OCP) but relatively preserved proteolysis (OHP) resulting in increased fibrinolysis (overall fibrinolytic potential (OFP) (Table 1). Normal controls ≥ 50 years old had more prothrombotic TEG (particularly reduced clot lysis) with increased fibrin generation and proteolysis. The impact of age on CAT was minimal. East Asians ($n=31$) had lower endogenous thrombin potential on CAT ($p < 0.01$) compared to participants with European origin ($n=54$) with minimal differences seen on TEG and fibrin generation. Interestingly, there were 2 distinct patterns of CAT curves with the concave/flattened type being more common in males (11/31 vs 12/65 females) and in those with poor lipid profile. There was no correlation seen with TEG and fibrin generation, however, these patients had higher P-selectin (55.8 vs 48.4 ng/mL, $p=0.03$).

Conclusion: This study highlights the differences seen in gender, age and ethnicity on global coagulation assays within our normal population, as well as unique patterns seen with different global coagulation assays. Further studies are required to assess the clinical significance of these differences.

	Male	Female	p-value	Age <50years	Age ≥ 50 years	p-value
No of controls	31	65		55	41	
TEG parameters						
R time (min)	8.4	6.9	0.03	8.1	6.4	0.01
K time (min)	2.3	2.3	<0.01	2.9	2.1	<0.01
α -angle (°)	51.2	59.4	<0.01	51.7	62.8	<0.01
Max amplitude (mm)	54.3	59.5	<0.01	56.1	60.1	0.01
LY30 (%)	1.9	2.1	0.82	3.0	0.0	0.01
CAT parameters						
Lag Time (min)	3.1	3.2	0.41	2.9	3.6	<0.01
ETP (nM.min)	1258.0	1390.2	0.02	1336.2	1362.8	0.62
Peak height (nM)	193.2	236.7	0.11	219.1	227.3	0.55
Vel Index (nM.min)	56.5	80.5	0.01	74.8	70.1	0.54
OHP parameters						
OCP	56.1	61.4	0.04	54.7	66.8	<0.01
OHP	29.5	29.2	0.85	26.6	33.0	<0.01
OFP (%)	46.8	51.5	0.01	49.5	50.6	0.55
P-selectin	54.2	48.0	0.03	54.6	47.1	0.01

479. Outpatient management of deep vein thrombosis using direct oral anticoagulants is safe and cost-effective

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Aim:

Direct oral anticoagulant (DOAC) is increasingly the treatment of choice for venous thromboembolism given its ease of use compared to warfarin. To ensure safe prescribing of DOAC and prevent delay of supply for dischargeable patients with deep venous thrombosis (DVT) presenting to Emergency Department (ED) outside pharmacy hours, we implemented a "Rivaroxaban after-hours pack" system, which requires prescribers to obtain approval through *GuidanceMS* prior to pack provision. We aim to review the safety and cost-effectiveness of this program.

Method:

A retrospective evaluation of DVT patients discharged from ED with Rivaroxaban after-hours pack from April 2015 to December 2016, with minimum follow-up of 6 months. Data collected included baseline demographics, investigations, medication history, potential contraindications and length of stay.

Results:

Seventy-seven patients with newly diagnosed DVT (median age 52 years (22-84)) were discharged from ED with Rivaroxaban after-hours pack. 98.7% of the patients met the weight criteria of safe prescribing (50-120kg) and all patients had eGFR >30 mL/min. Of 67 patients (87.0%) who had liver function testing done, one did not meet the criteria. Two patients (2.6%) were known to have active malignancy at time of prescribing and no patients had mechanical valve or antiphospholipid syndrome. All patients received pharmacist follow-up via telephone conversation for education with referral to Thrombosis Outpatients. One patient re-presented with clinically significant bleeding (1.3%) and two patients had clot extension (2.6%) while on rivaroxaban despite reportedly good compliance. There was marked improvement in length of stay compared to DVT patients audited during the warfarin era (8.0 hours vs 5.17 days, $p < 0.001$).

Conclusion:

All DVT patients were safely prescribed and given Rivaroxaban through this system, with significant reduction in length of stay in hospital without increased bleeding rates. This system is efficient and cost-effective in ensuring safe provision of Rivaroxaban outside of pharmacy hours.

480. Age-adjusted cut-off using the IL D-dimer HS assay to exclude pulmonary embolism in emergency patients

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Background/Aim

The ADJUST-PE study showed that an age adjusted D-dimer cut-off (age years X 10ng/ml if >50 years) combined with a non-high pretest probability (PTP) can increase the proportion of older patients in whom pulmonary embolism (PE) can be safely excluded. The IL D dimer HS assay was not assessed in this study. To evaluate the efficiency (proportion of patients in whom PE could be excluded based on a combination of non-high PTP and negative D-dimer) of an age-adjusted cut-off compared with other cut-offs using this assay.

Methods

We performed a retrospective analysis of consecutive patients presenting with symptoms of acute PE to one of three Monash Health Emergency Departments (January 2013-January 2014) who had both D dimers and Computer Tomography Pulmonary Angiography (CTPA) and calculated their Wells score. We determined efficiency rates for patients with non-high PTP using four cut-offs: (i) age-adjusted (ii) current laboratory (200 ng/ml), (iii) conventional (230ng/ml), and (iv) modified (375ng/ml if age ≥60 years)

Results

One hundred and seventy six patients were included (mean age 58.5 years; 54.0% males; 71.0% age >50 years). Prevalence of PE in the overall, non-high and high PTP groups were 17.0%, 13.0%, and 24.6% respectively. In the non-high PTP group (115 patients), efficiency for the age-adjusted, current, conventional, and modified cut-offs were 37.4% (95% CI 29.1,46.5), 9.6% (95% CI 5.4,16.3), 24.3% (95% CI 17.4,32.9) and 30.4% (95% CI 22.8,39.4) respectively. Corresponding false negative rates were 13.3 (3.7,37.9), 0 (0,20.4), 6.7 (1.2,29.8) and 6.7 (1.2,29.8) respectively.

Conclusion

The increase in efficiency of an age-adjusted cut-off over the conventional cut-off using the IL HS D-dimer assay for PE exclusion appears modest 13.0% (95% CI 0.5, 25.1) with a potential loss in sensitivity. There is currently insufficient prospective data to employ an age adjusted D-dimer management strategy for PE exclusion using this assay.

481. The role of thromboelastography G Parameter in the prediction of acute coronary syndrome

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Objective:

To investigate the early prognostic value of thromboelastography G (TEG-G) parameter in patients with acute coronary syndrome (ACS).

Methods: A total of 160 patients with ACS, 52 patients with stable angina pectoris (SAP) as control were enrolled. Baseline characteristics and clinical history of the two groups were recorded. Routine blood test, cardiac markers, lipid profile, HA1C, routine coagulation tests and TEG were determined. Receiver operating characteristic (ROC) curve was used to evaluate the diagnosis performance of each index. Binary logistic regression model was used to define the independent risk factors of ACS.

Results: No significant differences were detected between groups for age, men gender, BMI, blood pressure, lipid profile, HA1C, previous MI/CIS history, previous PCI/CABG history and dyslipidemia. Patients with ACS exhibited higher prevalence of diabetes and the greater prevalence of hypertension than patients with SAP. cTNI level in stable patients measured were significantly lower than ACS patients, while other biomarkers (AST, LDH, CK-MB and MYO) in the panel showed no statistically significance. Fibrinogen and platelet count levels were also significantly higher in ACS group. Compared to TEG parameters, all have significant difference between two groups, except R value. The area under ROC curve of TEG-G was 0.904. The optimal cut-off value for the diagnosis of ACS was 9.6 dyne/ cm², while the sensitivity was 80% and the specificity was 90.4%. In logistic regression model, TEG-G value was associated with ACS (odds ratio, 3.039; 95% confidence interval, 2.018-4.577) and TEG-G was an independent risk factor for ACS.

Conclusions: TEG-G reflects the physical strength of the clot including the fibrin network and the contribution of platelets to clot strength, which could be used as a predictor of activation of platelet and fibrinogen. Combined with cTNI, TEG-G is eligible to be a new biomarker for early diagnosis of ACS.

483. Immunogenicity Assessment of a Recombinant B-Domain–Deleted Porcine-Sequence FVIII in Patients With Acquired Haemophilia A

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Aim:

A recombinant B-domain–deleted porcine-sequence factor VIII (rpFVIII; OBIZUR) was recently approved for treatment of bleeding episodes in adults with acquired haemophilia A (AHA). We previously reported results for inhibitory antibodies against rpFVIII and human factor VIII (hFVIII) from a phase 2/3 clinical trial and evaluated their effect on treatment efficacy. The aim of this study was to extend these data by analyzing binding immunoglobulin (Ig) G and IgM antibodies against hFVIII and rpFVIII and evaluating their cross reactivity.

Methods:

The pivotal phase 2/3 study (NCT01178294) and the methods in principle for antibody analytics were previously reported (Whelan 2013; Kruse-Jarres 2015). 28 patients with AHA were evaluated for inhibitory antibodies against hFVIII and rpFVIII; 26 had supplementary samples that were analyzed for total binding IgG and IgM antibodies.

Results:

Consistent with AHA diagnosis, all patients evaluated (n=26) tested positive for binding IgG and inhibitory antibodies against hFVIII at screening. 20 of those patients showed positive results for binding anti-rpFVIII IgG (10/20 had inhibitory antibodies against rpFVIII) that can be considered cross-reactive anti-hFVIII antibodies. After treatment with OBIZUR, 10 patients were tested positive for binding IgG antibodies against rpFVIII. De novo inhibitory antibodies against rpFVIII were detected in 5 patients. Among those, 2 patients showed a concomitant decrease of anti-hFVIII IgG. This suggests that the observed anti-rpFVIII inhibitors resulted from an immunogenic response to rpFVIII.

Conclusion:

An increase in cross-reactive anti-hFVIII antibodies or a specific immunogenic response to rpFVIII could contribute to the development of antibodies against rpFVIII observed during the study. All subjects had a positive response to OBIZUR treatment within 24 hours after initiation. Further characterization of subclass and isotype distribution and affinities of anti-rpFVIII antibodies will provide additional insights into rpFVIII immunogenicity.

484. Obtaining an accurate platelet count in patients with pseudothrombocytopenia with fluoride oxalate collection.

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Pseudothrombocytopenia (PTCP) is an uncommon phenomenon in which *in vitro* platelet clumping results in spuriously low platelet counts on automated cell counters. Although pseudothrombocytopenia has no clinical impact, it is important to be aware of to avoid unnecessary investigation and inappropriate treatment. EDTA-dependent antibody mediated platelet agglutination is an important cause of pseudothrombocytopenia

Patient	EDTA	Citrate	Fluoride Oxalate	Lithium/Heparin
1	82	44	208	42
2	60	125	216	145
3	143	37	175	75

Techniques to obtain an accurate platelet count include collection and processing of blood at 37 degrees Celsius, and collection of blood in citrate tubes, however 10-20% of patients with EDTA mediated PTCP will also have platelet agglutination with citrate.

We present a case series of 3 well outpatients with PTCP occurring in both EDTA and citrate collection, who then had follow up simultaneous platelet counts performed in EDTA, citrate, heparin and fluoride oxalate tubes for comparison. The results are outlined in the table below. The results indicate the fluoride oxalate may be a readily available alternative to EDTA and citrate tubes to obtain a platelet count in patients with PTCP.

485. Fixed dose Prothrombinex-VF use in warfarin reversal

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Aim

Prothrombinex-VF is used for warfarin reversal in various clinical settings. The aim of this study is to evaluate the efficacy and safety of fixed dose Prothrombinex-VF compared to weight based dosing in a hospital setting.

Method

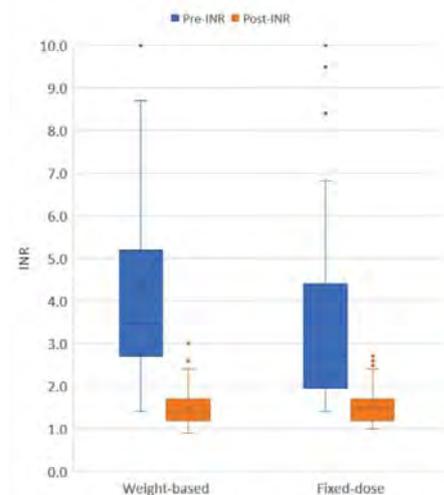
All patients who received Prothrombinex-VF for warfarin reversal between 1st January to 31st December 2016 were included. Patients who received 15IU/kg or less were labelled as having *fixed-dose* treatment, and those who received more than 15IU/kg were assigned to the *weight-based* treatment group. The primary outcomes were post-reversal international normalised ratio (INR), proportion of patients requiring repeat Prothrombinex-VF, and 30-day complication rates, including all-cause mortality, thrombosis and bleeding.

Results

One hundred and forty-four patients were analysed. Fifty-seven patients were in the *fixed-dose* group, and 87 were in the *weight-based* group. Baseline characteristics were balanced between the two groups. The median INR before reversal was 2.7 in the *fixed-dose* group and 3.6 for the *weight-based* group ($p = 0.004$). The median dose of Prothrombinex-VF given was 1000IU (average 10.9IU/kg) in the *fixed-dose* group and 1,500IU (average 24.2IU/kg) in the *weight-based* group. Following Prothrombinex-VF, the median INR was 1.5 for the *fixed-dose* group and 1.4 for the *weight-based* group ($p = 0.440$). 6 patients (10.5%) required a repeat dose in the *fixed-dose* group and 10 (11.5%) in the *weight-based* group ($p = 0.857$). 22 patients (15.3%) experienced complications (death, bleeding and/or venous and arterial thrombosis) within 30 days of warfarin reversal with 7 (12.3%) complications in the *fixed-dose* group and 15 (17.2%) complications in the *weight-based* group ($p = 0.418$).

Conclusion

Fixed dose Prothrombinex-VF appears to be as efficacious and safe as weight-based dosing in the setting of warfarin reversal.



486. Target Joint Outcomes with Prophylaxis with rFIXFc in Adults and Adolescents with Haemophilia B: Updated Results from B-YOND

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Background:

The B-YOND extension trial evaluates long-term safety and efficacy of rFIXFc in adults/adolescents with severe hemophilia B who completed the Phase 3 B-LONG study.

Aims:

To present longitudinal data outcomes from subjects with target joints at B-LONG entry through the 2nd interim data cut of B-YOND.

Methods:

Subjects completing B-LONG enrolled in 1 of 4 treatment groups in B-YOND: weekly prophylaxis (WP; 20-100 IU/kg every 7 days), individualised prophylaxis (IP; 100 IU/kg every 8-16 days), modified prophylaxis (MP; for subjects not achieving optimal dosing with IP or WP), or episodic treatment. Subjects with ≥ 1 target joint (major joint with ≥ 3 bleeding episodes in a 3-month period) at B-LONG entry were evaluated. A target joint resolved if there were ≤ 2 spontaneous bleeds in the target joint over 12 months. Outcomes were analysed over the cumulative duration of B-LONG through the second B-YOND interim data cut (11 Sep 2015).

Results:

Of 117 B-LONG subjects with on-study data, 60 had a total of 166 target joints at baseline. These subjects received a cumulative median (IQR) of 3.4 (1.4-4.2) years of rFIXFc. In subjects with target joints at baseline, on-study overall and target joint annualised bleeding rates (ABRs) with rFIXFc prophylaxis were lower than bleeding rates with prestudy (pre-B-LONG) prophylaxis or episodic treatment (**Table**). 37.5% of WP, 8.3% of IP, and 33.3% of MP subjects had no target joint bleeding episodes during B-LONG/B-YOND, and 100% (37/37) of subjects with evaluable target joints at B-LONG baseline (≥ 12 months follow-up; no target joint surgery ≤ 12 months) had target joints resolved. In the WP and IP groups, median (IQR) average weekly prophylactic dose was 45.2 (37.3-55.8) IU/kg and 64.7 (46.7-82.3) IU/kg, respectively; median (IQR) dosing interval in the IP group was 10.3 (8.9-13.2) days.

Conclusions:

Long-term rFIXFc prophylaxis resulted in low target joint ABRs and target joint resolution in all adults/adolescents with evaluable target joints at baseline in all dose groups.

487. Longitudinal Modified Hemophilia Joint Health Scores (mHJHS) in Children, Adolescents, and Adults with Severe Hemophilia A With Long-term rFVIII Fc Prophylaxis

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Background: Hemophilic arthropathy is a challenging complication of hemophilia; prophylaxis has been shown to prevent chronic arthropathy.

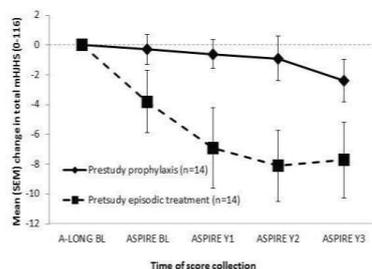
Aims: To evaluate longitudinal change in joint health with rFVIII Fc prophylaxis during A-LONG (adults/adolescents) and Kids A-LONG and the ongoing extension study, ASPIRE (NCT01454739).

Methods: Joint health was assessed by mHJHS at A-LONG/Kids A-LONG baseline (BL), ASPIRE BL and annually thereafter. A-LONG subjects with mHJHS data at A-LONG BL, ASPIRE BL, and ASPIRE Year 1 [Y1], Year 2 [Y2], and Year 3 [Y3] were included (Kids A-LONG BL, ASPIRE BL, Y1, and Y2 available for kids). Change from A-LONG/Kids A-LONG BL to last visit was analyzed using a paired t-test.

Results: Mean (SEM) mHJHS total score for adults/adolescents with data at all time points (n=28) was 25.0 (2.9) at A-LONG BL. Mean (SEM) change from A-LONG BL was -2.0 (1.2) at ASPIRE BL, -3.8 (1.5) at ASPIRE Y1, -4.5 (1.6) at ASPIRE Y2, and -5.0 (1.5) at ASPIRE Y3 ($P<0.01$). These improvements were observed regardless of prestudy regimen (**Figure**). Similar results were observed regardless of age or the presence/absence of BL target joints. Subjects were classified according to the median of mHJHS at BL. The half with higher BL mHJHS (>22) had greater mean (SEM) improvements vs A-LONG BL through ASPIRE Y3 (-8.4 [2.6]) than the half with lower initial mHJHS (-1.8 [1.2]). Mean (SEM) improvements at ASPIRE Y3 vs A-LONG BL were observed for both weight-bearing (-1.1 [0.5]) and non-weight-bearing (-3.0 [0.8]) joints. The mHJHS components that showed $\geq 25\%$ score reduction from A-LONG BL to ASPIRE Y3 were joint instability (-89%), swelling (-46%), joint pain (-31%), and muscle atrophy (-25%). Results from the Kids A-LONG cohort (n=24) also showed mean (SEM) improvement from A-LONG BL to ASPIRE Y2 (-1.2 [0.6]; $P<0.05$).

Conclusions: Among subjects receiving long-term rFVIII Fc prophylaxis, continuous annual improvement in mHJHS scores was observed, regardless of prestudy dosing regimen, even among those with reduced joint health at BL.

Figure. Mean (SEM) change from baseline in mHJHS total score and by prestudy regimen from A-LONG BL to ASPIRE Y3 in adults/adolescents.



488. Efficacy and Safety of rFVIII Fc Prophylaxis in Pediatric, Adolescent, and Adult Subjects with Severe Hemophilia A Over 3-4 Years: The ASPIRE Study

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Background: The ASPIRE extension study evaluates long-term safety and efficacy of rFVIII Fc in previously treated subjects with severe hemophilia A.

Aim: To present safety and efficacy data from the third interim data cut (Y3) of ASPIRE (11 Jan 2016).

Methods: Eligible subjects completing A-LONG (aged ≥ 12 y) received 1 of 4 treatments in ASPIRE: individualized prophylaxis (IP), weekly prophylaxis (WP), modified prophylaxis (MP), or episodic treatment (ET); or for those < 12 y who completed Kids A-LONG, IP or MP. Subjects could switch treatment groups at any time. The primary endpoint was occurrence of inhibitor development.

Results: Of 153 adults/adolescents who completed A-LONG, 150 enrolled in ASPIRE, and 78 remained on-study at Y3. Of 67 children who completed Kids A-LONG, 61 enrolled in ASPIRE and 45 remained on-study at Y3. No inhibitors were observed across age groups in ASPIRE. The safety profile of rFVIII Fc was consistent with the parent studies and prior interim analyses. Median (IQR) duration of treatment was 4.1 (3.0-4.2) y (A-LONG subjects) and 2.9 (2.3-3.1) y (Kids A-LONG subjects). Among A-LONG subjects, 19 (12.7%) changed treatment groups at least once. From the end of A-LONG (or Kids A-LONG), 70.3% (90.2%) had no change in dosing interval, while 23.4% (6.6%) extended and 6.3% (3.3%) shortened. Median (IQR) change in weekly consumption at ASPIRE Y3 was 0 (0-0) from end of A-LONG and 0 (0-3.2) from end of Kids A-LONG. Dosing characteristics (Table 1) and ABRs (Table 2) are summarized. Among adults/adolescents, 93.6% (IP), 97.8% (WP), 94.5% (MP), and 99.2% (ET) of bleeding episodes were controlled with 1-2 rFVIII Fc injections. Per subject, median doses to resolve bleeding episodes were 45.6 (IP), 34.1 (WP), 37.1 (MP), and 27.3 (ET) IU/kg. Results were generally similar for pediatric subjects.

Conclusion: The safety and efficacy of rFVIII Fc prophylaxis was confirmed over ~ 4 y in adults/adolescents and ~ 3 y in children. ABRs remained consistently low while maintaining extended prophylactic dosing intervals.

489. Long-Term Impact of rFVIII Fc Prophylaxis in Paediatric, Adolescent, and Adult Subjects with Target Joints and Severe Haemophilia A

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Background:

Long-term safety and efficacy of rFVIII Fc are being evaluated in the ongoing ASPIRE extension study of subjects with severe haemophilia A who completed A-LONG or Kids A-LONG.

Aim:

To present data on re-bleeds and target joint resolution from subjects with target joints at entry into the parent study through the third ASPIRE interim data cut.

Methods:

Subjects with ≥ 1 target joint (major joint with ≥ 3 bleeding episodes in a 6-mo period) at parent study entry with available pre-parent study data and on-study data were evaluated. ASPIRE treatment groups (≥ 12 y) were: individualised prophylaxis (IP), weekly prophylaxis (WP), modified prophylaxis (MP), or episodic treatment (ET); or (< 12 y) IP or MP. Outcomes were analysed over the duration of the parent study through the third ASPIRE interim data cut (11 Jan 2016).

Results:

113 A-LONG subjects had target joints at baseline; 111 with evaluable data had 287 target joints at baseline and a cumulative median (IQR) 4.0 (2.8, 4.1) y on rFVIII Fc. 13 Kids A-LONG subjects had 15 target joints at baseline and 3.0 (0.5, 3.1) y on rFVIII Fc. Target joint annualised bleeding rates were low for subjects on rFVIII Fc prophylaxis (**Table 1**). 43.9% of IP, 42.3% of WP, and 6.3% of MP A-LONG subjects and 53.8% of Kids A-LONG subjects (all IP) had no target joint bleeding episodes. Among prophylaxis subjects with target joints at baseline and 12 mo follow-up, 100% of A-LONG and Kids A-LONG subjects had ≥ 1 target joint resolved (≤ 2 spontaneous bleeding episodes in 12 consecutive mo); 99.6% and 100% of evaluable target joints in A-LONG and Kids A-LONG subjects were resolved, respectively. **Table 2** shows prophylactic dose and dosing intervals. In adults/adolescents, 96.4% of target joint bleeding episodes were controlled with ≤ 2 rFVIII Fc injections; patients rated 82.0% of injections to control a bleeding episode as excellent or good.

Conclusion:

Low target joint ABRs and effective target joint resolution occurred in children, adolescents, and adults on long-term rFVIII Fc prophylaxis.

490. Evaluation of global coagulation assays in patients with risk factors for cardiovascular disease

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Aim: Predicting an individual's cardiovascular disease (CVD) risk remains challenging and we aim to evaluate the use of global coagulation assays (thromboelastography, thrombin generation using calibrated automated thrombogram (CAT) and fibrin generation with overall haemostatic potential (OHP) assay) in patients at-risk of CVD; as well as identify the differences compared to normal controls and between different at-risk groups.

Method: This prospective pilot study recruited patients with high-risk cardiovascular risk factors: chronic renal impairment, diabetes mellitus and/or, those with ≥ 2 traditional CVD risk factors (hypertension, hypercholesterolaemia, smoking, obesity, age > 65 years, family history of CVD). Fasting blood samples were collected for baseline investigations including full blood count, coagulation and lipid profile, as well as experimental testing with thromboelastography (TEG® 5000S) utilising citrated whole blood. Additional samples were double-centrifuged to obtain platelet-poor plasma for later assessment with CAT and OHP.

Result: Fifty-five patients (26 males; 29 females with median age 61 years) were recruited and the results were compared to age-matched normal controls. High-risk CVD patients had more prothrombotic TEG® parameters with increased maximum amplitude and markedly reduced clot lysis ($p < 0.001$) when compared to normal controls, with increasing significance with higher Framingham scores. Interestingly, the endogenous thrombin potential (1242.2 vs 1363.0 nM/min; $p = 0.01$) and thrombin peak (199.6 vs 227.3 nM; $p = 0.02$) are paradoxically reduced while there is no difference in the OHP findings. Aspirin and statin use were not associated with significant difference in the parameters.

Conclusion

Patients at-risk of CVD demonstrated increased clot strength with reduced lysis on whole blood TEG, but interestingly there was an inverse association with platelet-poor thrombin generation parameters. This paradoxical finding may be due to compensatory mechanisms within the Virchow's triad and may represent a useful biomarker in CVD. A larger prospective study is being conducted to confirm these findings and identify the underlying pathophysiology.

	Control (n=41)	High-risk CVD (n=55)	p-value
TEG parameters			
R time (min)	6.4 (5.4 – 7.5)	8.3 (7.5 – 8.9)	<0.001
Alpha-angle (°)	62.8 (□ 7.2)	52.8 (□ 9.5)	<0.001
Maximum amplitude (mm)	60.1 (□ 5.5)	69.3 (□ 6.3)	<0.001
LY30 (%)	0.9 (0.1 – 1.1)	0.0 (0.0 – 0.4)	0.001
CAT parameters			
Lag time (min)	3.4 (3.0 – 4.1)	3.7 (3.3 – 4.5)	0.07
Endogenous thrombin potential	1363.0 (± 216.0)	1242.2 (□ 217)	0.01
Peak thrombin	227.3 (± 61.1)	199.6 (□ 42.6)	0.02
Velocity index	70.1 (± 29.8)	60.1 (□ 19.9)	0.06
OCP parameters			
Overall coagulation potential	66.7 (± 8.6)	65.6 (□ 12.6)	0.61
Overall haemostatic potential	33.0 (± 7.0)	33.1 (□ 10.6)	0.96
Overall fibrinolytic potential	50.6 (± 8.3)	49.5 (□ 11.3)	0.62

492. Platelet Type Von Willebrand Disease: A journey from diagnosis to pregnancy.

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Background:

Platelet type Von Willebrand's Disease (PT-VWD) is a rare autosomal dominant bleeding disorder, caused by a gain of function mutation in the GPIBA gene, leading to hypersensitive binding between the platelet GPIBA receptor and HMW VWF multimers. The Ristocetin Induced Platelet aggregation (RIPA) assay including mixing study and genetic testing can be used to confirm the diagnosis. This differentiates between the phenotypically similar Type 2B VWD, which is critical in managing bleeding episodes such as pregnancy.

Case report:

We describe the case of a 34 year old female who was referred to our haematology clinic after a moderate post-partum haemorrhage, long standing menorrhagia and autoimmune hypothyroiditis: Platelet count $77 \times 10^9/L$; VWAg 56%; RicoF: 24%; CBA: 25%. RIPA showed enhanced aggregation at 0.1mg/mL with mixing studies leading to preliminary diagnosis of PT-VWD. On referral to our haematology antenatal clinic later at 12/40 gestation, genetic testing showed heterozygosity for the missense variant c.745G>A (pGly249Ser), confirming the diagnosis. Her platelet count and VWF levels were monitored throughout her pregnancy: Platelet count decreased from 114 to $77 \times 10^9/L$; VWF levels increased, with VWF ratios >0.9 by 23/40 gestation. Prednisolone therapy during her post-partum bleed increased her platelet count from 107 to $151 \times 10^9/L$. In the treatment plan cryoprecipitate and VWF concentrates were excluded as the addition of HMW VWF multimers may cause increased clearance of platelets. Fibrinogen concentrate was to be used in the event of a haemorrhage. The female neonate was delivered without complication and with a normal platelet count. We later tested her and found her to be heterozygous for pGly248Ser mutation. The simplified RIPA mixing assay enabled the preliminary diagnosis of PT-VWD prior to confirmatory genetic testing. Although thrombocytopenia is associated with PT-VWD, role of co-existing autoimmunity could not be excluded.

493. The Innocent Bystander: Lupus Anticoagulant interference in one stage clotting assays in a preoperative child.

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Introduction:

Lupus anticoagulant (LAC) has been reported in children and its presence has been reported as transient in the majority of the cases. Less is known about the prevalence of LAC in children and its clinical significance.

Case report:

A 4 year old male presented to the emergency department with rectal bleeding and suspected intussusception on clinical examination. There was no significant past medical history and specifically, no personal or family history of a bleeding disorder. Full blood count and routine biochemistry at admission was unremarkable.

Coagulation profile showed PT 14.2 secs (RR: 14.4-16.4 secs), APTT 99 secs (RR: 34-45 secs) and Fibrinogen 2.4g/L (RR: 1.9-3.7g/L). Heparin resistant APTT showed no evidence of heparin contamination and the results were confirmed on repeat. With emergency surgery thought likely, mixing studies, factor VIII and IX assays were performed. The mixing studies showed an APTT correction of 43% and concentrations of factor VIII of 0.19 IU/mL and factor IX of 0.37 IU/mL.

Further testing was undertaken to discriminate between a specific factor inhibitor and a LAC which would have major implications for bleeding risk during surgery. Dilute Russell Viper Venom test Time (DRVVT) was prolonged and showed phospholipid dependence with a normalised screen/confirm ratio of 1.87 consistent with presence of LAC. Anti β -2 glycoprotein antibodies were not detected.

The Patient ultimately did not require surgical intervention and was successfully managed conservatively. A repeat test has been recommended to ascertain persistence.

Conclusion:

While uncommon, the case highlights the possibility of lupus anticoagulant in paediatric population and the potential interference in one stage clotting assays.

494. Procoagulant effects of platelet activation and its inhibition by colchicine

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Aim.

Platelets play an important role in diseases such as cardiovascular disease and cancer, especially through their release of microparticles. We aimed to examine the effects of low-level and strong platelet activation on microparticle release, the effect of microtubule inhibition by colchicine on these microparticles, and the role of these microparticles in global coagulation.

Method.

Citrated platelet-rich plasma (PRP) from healthy donors was incubated with 2 mM colchicine or vehicle (water) at 37°C for 30min. PRP was then incubated with 20 µM ADP or 10 µM epinephrine for a further 30min at 37°C. Platelets were removed and the resulting plasma stored at -80°C. Microparticle levels were measured by binding of labelled lactadherin (Lact.+), CD61 and CD62P in a flow cytometry assay. Global coagulation was assessed by the overall haemostatic potential (OHP) assay and the effect of microparticles on coagulation assessed by removal via ultracentrifugation, and via a modification (mOHP) in which plasma was diluted in microparticle-depleted plasma.

Result.

The conditions used for incubation caused mild platelet activation. Colchicine attenuated both this and agonist-induced platelet activation (Fig 1A). Elevation of Lact./CD61+ and CD62P+ microparticles in the plasma was also attenuated by prior colchicine incubation (Fig 1B). The plasma showed shortened delay to fibrin generation (Fig1C) and increased mOHP parameters. Removal of microparticles from plasma abrogated fibrin generation by the OHP assay. OHP results correlated with elevated levels of Lact.+ /CD61+ (Fig1D) and CD62P+ microparticles.

Conclusion.

These studies provide novel insights into the links between platelet activation, microparticles and global coagulation and demonstrate modulation by a microtubule inhibitor.

495. Safety and efficacy of long-term open-label dosing of subcutaneous romiplostim in children with Immune Thrombocytopenia

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Background:

Children with ITP for ≥ 6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in an open-label long-term extension study, data from which is presented here.

Methods:

Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 $\mu\text{g}/\text{kg}$ for patients previously receiving placebo, adjusted from 1–10 $\mu\text{g}/\text{kg}$ to target platelet counts of 50–200 $\times 10^9/\text{L}$. Incidence of adverse events (AEs) was the primary endpoint.

Results:

As of 24 Feb 2016, 66 patients entered the extension study; 65 received romiplostim for up to 6.2 years. At baseline, median (min-max) age was 11 (3–18) years; 9.1% had prior splenectomy. Median (min-max) baseline platelet count was 27.5 (2–458) $\times 10^9/\text{L}$. All 65 patients received their doses per protocol $>90\%$ of the time. Twenty-two patients discontinued the study. Fifty-two serious AEs (3 treatment related) occurred in 17 patients. Bleeding AEs occurred in 56 patients (5 treatment related). Bleeding AEs occurring in ≥ 10 patients included contusion, epistaxis, petechiae, and gingival bleeding. No thrombotic events were reported. There were no peripheral blood abnormalities to warrant a bone marrow examination. No patients had anti-TPO neutralizing Ab. From week 2 on, median platelet counts remained $>50 \times 10^9/\text{L}$; platelet counts were $>100 \times 10^9/\text{L}$ at most timepoints, despite an observed decrease in the median dose from 4–5 $\mu\text{g}/\text{kg}$ to 2–3 $\mu\text{g}/\text{kg}$ around week 160. Nearly all (94%, 61/65) patients had a platelet response. Nine (14%) patients entered remission (table).

Conclusion:

Over 6 years of data show that $>90\%$ of children achieved a platelet response with romiplostim, most responding $\geq 75\%$ of the time. The safety profile was overall tolerable, similar to that in past studies. Some children (9/66) with longstanding ITP entered remission after receiving romiplostim.

496. Outcomes in Patients with Haemophilia undergoing total joint replacement.

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Aim: Our aim is to see if patients with haemophilia undergoing total joint replacement have more complications and poorer outcomes than the general population, why this is and how we can improve outcomes for the future.

Method:

The Christchurch Haemophilia service database was searched to identify all patients with Haemophilia A or B. These patients were searched on the online health portal, Health Connect South, to ascertain if they had had any joint replacements and collect data accordingly.

Data that was collected included gender, date of birth, age, joint(s) that were replaced, reason for joint replacement, pre op consult with haematology and haemostasis plan written, intra operative complications, pre and post op haemoglobin level, need for transfusion, length of hospital stay, long term complications i.e. need for further operations and long term orthopaedic outcomes.

These outcomes were then compared against an age and operation matched control group.

Result:

Results so far show that planning surgery with Haematology support is crucial for good outcomes including pre-emptively inserting PICC lines for factor administration. It is also clear that this patient group undergoes joint replacement at a much younger age and that in a particular case noncompliance from the patient led to poorer outcomes and highlights the importance of constant patient education.

Conclusion:

This study highlights the importance of peri-operative planning and clear and concise haemostasis protocols. It is also important for advance insertion of PICC lines so as factor infusions are not interrupted which could result in overall poorer outcomes. From a particular case it highlighted the importance of patient education and adherence to treatment. From this study we have produced a surgical planning pathway specifically for patients with Haemophilia to help guide surgical teams in this complex patient group.

497. Australian Frequency of Polymorphisms Clinically Relevant for Diagnosis of Type I von Willebrand's Disease

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Aim:

Diagnosis of type 1 von Willebrand's Disease (vWD) is currently based on bleeding history in combination with levels of von Willebrand Factor (vWF) antigen and activity in the blood. However, the common laboratory assays used for such diagnoses can be affected by inherited single nucleotide polymorphisms (SNPs) in the vWF gene. Occurring in the A1 domain of vWF, the D1472H polymorphism interferes with the vWF:RCO assay, while the A1381T effects the vWF-GPIIb interactions. Population studies have shown that 17% of healthy American Caucasians carry D1472H, and 65% of Europeans are positive for A1381T. However, the frequency of these SNPs in the Australian population has not been reported. Therefore, the aim of this study was to determine the prevalence of D1472H and A1381T polymorphisms in the Australian population with a view to provide better laboratory-based diagnostic practices.

Methods:

Australian frequencies of D1472H and A1381T were inferred by genotype imputation using gene mapping data from the Hunter Cohort Study (n=1717) using the 1000 Genomes and HapMap reference panels, respectively. The results were then validated in a subset of samples by standard PCR-RFLP and qPCR methodologies.

Results:

In our Australian cohort, 12% and 64% of people were predicted to be carriers for the D1472H and A1381T polymorphisms, respectively. As such, the minor allele frequencies were estimated to be 0.06 ($R^2=0.72$) for D1472H and 0.40 for A1381T ($R^2=0.78$). Concordance with the Hunter Cohort samples was excellent at 100% for D1472H and 97% for A1381T. Both assays worked well and have now been established in our laboratory.

Conclusion:

Both D1472H and A1381T polymorphisms were found to be common enough in the Australian population to have a significant impact on methods used for diagnosis of VWD. Future studies will investigate the impact of these polymorphisms in patients previously diagnosed with vWD.

No conflict of interest to disclose.

499. Targeting the platelet internal membrane reveals a novel approach for improved anti-platelet therapies

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Background:

The class II PI3K, PI3KC2 α , is a broadly expressed lipid kinase with emerging biological roles. We have recently shown that PI3KC2 α is important in platelet structure and function using a mouse genetic approach - PI3KC2 α -deficient mice have impaired thrombotic function that appears due to a dysregulated open canalicular system (OCS) structure (Mountford, Nat Comm 2015). We have subsequently developed a novel pharmacological inhibitor of PI3KC2 α , X151, allowing us to examine whether a similar role for PI3KC2 α exists in human platelets.

Aim:

To determine the role of PI3KC2 α in the regulation of human platelet membrane structure and function, and the viability of targeting PI3KC2 α as an anti-thrombotic strategy.

Methods:

The structural impact of PI3KC2 α deficiency and inhibition on the OCS was assessed using TEM in 2D, and FIB-SEM in 3D. Standard SEM was used to examine OCS surface openings. The impact on platelet membrane function was examined in both static and dynamic adhesion assays. Ex vivo thrombosis in human whole blood, and in vivo thrombosis in mouse models, were used to assess impact on prothrombotic function.

Results:

PI3KC2 α inhibition with X151 in human platelets caused enlargement of the OCS leading to an increased OCS volume, similar to the effect seen in PI3KC2 α -deficient mouse platelets. An increase in the number and size of OCS openings at the plasma membrane was also observed. PI3KC2 α inhibition impaired platelet tether formation in flowing whole blood, and significantly reduced thrombus formation in an ex vivo human whole blood model and two distinct in vivo mouse models.

Conclusion:

These findings demonstrate that PI3KC2 α is involved in the regulation of platelet membrane structure and function in both mouse and human, and suggest that targeting the platelet membrane via PI3KC2 α may provide potential as a novel anti-thrombotic drug target.

500. Inter-laboratory validation of apixaban levels in ex-vivo patient samples using a modified anti-Factor Xa assay

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Background:

Laboratory monitoring of apixaban is not routinely required, but is useful in several situations (Table 1). In the absence of mass spectrometry, apixaban quantitation is performed with chromogenic anti-Xa assays using apixaban-specific calibrator. Sensitivity for apixaban detection is limited in routine coagulation tests,^{1,2} varies depending on the reagents used,³ and differs for *in-vitro* apixaban-spiked plasma, which is used in the majority of published studies, compared to *ex-vivo* patient plasma.⁴

Aims:

1. Determine apixaban levels (using a modified anti-Factor Xa assay with apixaban-specific calibrator) using **ex-vivo** patient samples and validate the reproducibility of this assay in three laboratories.
2. Assess the correlation between plasma apixaban level and prothrombin time (PT), activated partial thromboplastin time (APTT), dilute Russel viper venom time (DRVVT) and fibrinogen level using different reagents and assays on different analysers.

Method:

We have to date collected 40 blood samples from a planned sample size of 50 patients taking apixaban for >3days. Plasma was aliquoted into 3 equal volumes for the 3 laboratories. Apixaban levels were assessed after a single freeze-thaw cycle with an anti-Xa assay using Stago RUO apixaban calibrators with different reagents and analysers. Other coagulation tests were performed as per each individual laboratory protocol. After completing recruitment, simple descriptive statistics will be applied to baseline patient variables. For each PT reagent (thromboplastin), as well as APTT, DRVVT and fibrinogen, we will assess correlation with plasma apixaban levels using linear regression analyses and the Pearson correlation coefficient.

Results:

The majority of our cohort was taking apixaban for treatment or prevention of venous thromboembolism. Demographic data (Table 2) and apixaban levels performed at the collecting institution (Table 3) are shown. We intend to reach the sample size of 50 prior to performing inter-laboratory validation.

Conclusion:

Apixaban levels at the recommended doses show wide inter-individual variation. After collecting a further 10 samples, we will present the results of the inter-laboratory reproducibility of a modified anti-Xa assay for determining apixaban levels.

Table 1: Potential indications for apixaban levels	
Patient compliance	
Treatment failure	
Bleeding complications	
Safety for emergent surgery	
Potential variability in pharmacokinetics	
Extremes of body weight	
Impaired renal function	
Malabsorption	
Drug interactions	

Table 2: Demographic characteristics	
Gender	
Male [n]	19 (47.5%)
Female [n]	21 (52.5%)
Age	
Median	62.2 years
Range	18-85 years
Weight	
Median	83.0 kg
Range	50-130 kg
Creatinine Clearance (Cockcroft-Gault)	
Median	94 ml/min
Range	39-136 ml/min

Table 3: Apixaban levels			
Apixaban dose	2.5mg BD	5mg BD	All patients
n (%)	21 (52.5%)	19 (47.5%)	40 (100%)
Time since ingestion (hours)			
Median	6.18	5.17	5.7
Range	2.85-8.75	0.33 -10.17	0.33-10.17
Apixaban level (collecting institution) (ng/ml)			
Median	67	167.2	114.58
Range	14.8-331	24.4-590.9	14.8-590.9

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501. Anthocyanin supplementation in alleviating thrombogenesis in overweight and obese population: A randomized, double-blind, placebo-controlled study

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The aim was to evaluate the effect of anthocyanin (ACN) supplementation, the main component in coloured rice, in reducing thrombogenesis and maintaining hemostasis in pro-thrombotic overweight and obese individuals. Twenty-six (M = 9, F = 17) overweight/obese (BMI > 25) individuals participated in this randomized, double-blind, placebo-controlled, crossover design dietary intervention trial. Volunteers consumed ACN (320 mg/day) or placebo capsules for 28-days followed by a two-week wash-out period. ACN supplementation inhibited adenosine diphosphate (ADP)-induced platelet activation-related conformational change and degranulation by reducing PAC-1 expression by 12% and P-selectin expression by 9% respectively. ACN supplementation also alleviated thrombogenic progression by reducing monocyte-platelet aggregate formation by 29% and platelet endothelial cell adhesion molecule-1 (PECAM-1) expression by 21%. Platelet aggregation induced by ADP, collagen and arachidonic acid was reduced by 36%, 17%, and 24% respectively. ACN supplementation has the potential to reduce the risk of thrombosis in overweight/obese population by targeting specific pathways of platelet activation/aggregation and endothelial dysfunction associated leucocyte migration.

No conflict of interest to disclose.

502. Safety and efficacy data of recombinant FXIII in patients with congenital FXIII A-subunit deficiency

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Aim:

Recombinant FXIII (rFXIII) represents a new treatment opportunity for congenital FXIII A-subunit-deficient patients. Monthly prophylaxis with 35 IU/kg rFXIII controlled bleeding effectively, with an excellent safety profile¹. rFXIII exhibited first-order elimination, with a geometric mean half-life of 13.6 days, and a mean trough activity level of >0.1 IU/mL in all patients². The PK profile supports prophylactic monthly dosing with a fixed 35 IU/Kg regimen for all patients (regardless of age)^{3,4}. Extensive interim results are presented from mentorTM2, an ongoing phase 3b safety extension to the pivotal mentorTM1 trial.

Methods:

Patients received 35 IU/kg rFXIII every 4th week. FXIII activity was measured with the Berichrom[®] FXIII activity assay.

Results:

Sixty patients (34 from mentorTM1; 26 new) had 2,157 exposures (monthly dosing), corresponding to 168 patient years. The annual bleeding rate (ABR) was 0.042, 0.012 and 0.030 bleeds/patient/year overall, for spontaneous and for traumatic bleeds, respectively.

Six patients experienced 7 bleeds requiring FXIII treatment (5 trauma-induced; 2 spontaneous); of these, 1 trauma-induced muscular bleed was treated with rFXIII with excellent haemostatic response. No intracranial, internal organ or severe gastrointestinal bleeds occurred.

Mean FXIII trough levels were >0.1 IU/mL in all patients.

No thromboembolic events, fatal adverse events (AEs) or AEs leading to withdrawal were reported. Serious AEs were few (16 in 10 patients), and evaluated as unlikely related to trial drug. No anti-rFXIII antibodies were detected.

Conclusion:

rFXIII prophylaxis in the mentorTM program has demonstrated very effective bleed control, with an excellent safety profile. The ABR in mentorTM2 was lower than in mentorTM1 (0.138), and corresponded to an average patient having 1 bleed ~every 24 years. These efficacy data, combined with comprehensive PK and safety data, represent the largest collection in congenital FXIII A-subunit deficiency, and provide extensive evidence for the safety and efficacy of monthly prophylaxis with 35 IU/kg rFXIII.

503. Paradoxical Embolism- A Rare Cause of Acute Limb Ischaemia

Twomey B¹

¹St Vincent's Hospital, Melbourne, Australia

Introduction:

Paradoxical embolism was first reported by Cohnheim in 1877 and refers to an embolic phenomenon that originates in the venous vasculature and transverses into the arterial circulation through an intracardiac or pulmonary shunt. The most common intracardiac defect associated with paradoxical embolus is a patent foramen ovale. Clinical consequences of paradoxical embolus include acute limb ischaemia, stroke, intestinal infarction and myocardial infarction.

Case:

We present a case of paradoxical embolism causing acute lower limb ischaemia in the setting of pulmonary embolism and deep vein thrombosis. The patient was successfully treated with a thrombectomy and anticoagulation.

Conclusion:

Paradoxical embolism and intracardiac or pulmonary shunt should be immediately considered in a patient with venothromboembolism and acute limb ischaemia.

504. Inferior Vena Cava Hypoplasia- A Rare Cause of Deep Vein Thrombosis

Twomey B¹

¹St Vincent's Hospital, Melbourne, Australia

Introduction:

Inferior vena cava aplasia and hypoplasia are rare vascular anomalies that are hypothesised to be either congenital resulting from the embryological failure of the primitive veins to form the mature inferior vena cava or acquired secondary to early inferior vena cava thrombus in the intrauterine or perinatal period. The absence of the inferior vena cava is identified an important risk factor contributing the development of deep vein thrombosis, especially in young patients.

Case:

We present a case of inferior vena cava hypoplasia, combined with a horseshoe kidney in a 23-year-old man who initially presented with symptoms of bilateral lower limb deep vein thrombosis.

Conclusion:

In younger patients presenting with deep vein thrombosis, inferior vena cava aplasia or hypoplasia could be a predisposing factor and should be considered in the list of differential diagnosis.

506. Evaluating the efficacy of tranexaemic acid among hereditary bleeding disorder patients undergoing minor elective procedures

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Aim:

To evaluate the efficacy of tranexaemic acid (TXA) without prophylactic factor concentrate to prevent bleeding in hereditary bleeding disorders (HBD) patients undergoing minor elective procedures.

Method:

Retrospective review involving 64 patients (77 procedures) from January 2016 to February 2017. Patient demographics, HBD, severity, procedure type, use of TXA ± factor concentrates and bleeding outcomes within 30 days of procedure were recorded. Bleeding was assessed using WHO Bleeding Scale.

Results:

Of the 64 patients, 13 patients were lost to follow up, 3 patients received factor concentrates and TXA, 2 patients received only factor concentrates.

Table 1. Patients demographics who received TXA only.

Number of patients	46	Procedure type	
Male	38 (83%)	Gastrointestinal endoscopy without biopsy	12
Median age (years)	49 (18-89)	Gastrointestinal endoscopy with biopsy	11
Disease type		Dental procedures	27
Non-severe Haemophilia A	23	Cystoscopy	2
Severe Haemophilia A	5	Pilonidal sinus surgery	1
Non-severe Haemophilia B	7	Core biopsy breast	1
Severe Haemophilia B	4	TXA dose	1g TDS
Type 1 VWD	4	TXA median duration (days)	7 (2-21)
Type 2 VWD	1		
Platelet dysfunction disorder	2		
Regular prophylactic factor	9		

Table 2. Patients who developed bleeding.

Patient no.	Age	HBD	Procedure	Day of bleeding post procedure	Severity of bleeding	Management
1	74	Mild Haemophilia A	Dental extraction	14	Grade 1	Further 7 days TXA
2	22	Moderate Haemophilia A	Dental extraction	7	Grade 1	Further 7 days TXA
3	80	Mild Haemophilia B	Dental extraction	4	Grade 1	Further 4 days TXA
4	31	Severe Haemophilia B	Dental extraction	8	Grade 1	Further 1 day TXA

Conclusion:

Most HBD patients who undergo minor procedures can receive TXA only without needing factor concentrate. This study supports previous findings from our institution.

507. A retrospective cross-sectional study of overall survival for patients with Thrombotic Microangiopathy (TMA)

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Aims:

To study the demographic and outcome data of Thrombotic Microangiopathy (TMA) in relation to ADAMTS-13 activity in Malaysia.

Methods:

The TMA cases (year 2012-2016) are traced through the Ampang hospital medical records and matching with the National Registration Department for outcome. The data is analyzed using SAS (Statistical Analysis System) version 9.4.

Results:

243 cases with median age of 34.2 years and female predominance (57.6%). The 25-44 years is the most prevalent age group (34.1%); followed by 45-64 years (27.2%), 15-24 years (19.7%), 0-14 years (12.8%) and ≥ 65 years (6.2%). The three main ethnic distribution namely the Malay-62.5%, Chinese-23.5%, Indian-8.6% and others-5.4%. In ADAMTS-13 inhibitor cases, 32.1% is positive, 6.7% is borderline positive and 61.2% is negative. There is 20.9% of ADAMTS-13 activity $< 10\%$ for TTP. 72.7% of TTP is acquired while 22.7% is congenital. There are 55 (22.6%) mortality cases. Patients with ADAMTS-13 activity $\geq 5\%$ is associated with lower overall survival and the odd of death is 4 times higher compared to those with ADAMTS-13 activity $< 5\%$ (OR: 4.1327, $p=0.0425$, 95% CI: 0.9458, 18.0571). Most common TMA is secondary causes (42.6%), followed by primary acquired TTP (16.6%), aHUS/HUS (12.7%) and congenital TTP (6.4%). The secondary causes are SLE (34.9%), infection (33.7%), pregnancy (11.6%), transplant (9.3%), and malignancy (7%). TMA with secondary causes is associated with inferior outcome ($p=0.0387$) and transplant related TMA has the worst outcome ($p=0.0016$). The treatment received in the sub-analysis of 69 cases with complete treatment data is TPE (mean-8.4 cycles), methylprednisolone (85.5%), rituximab (36.2%), vincristine (26.1%), IVIG (14%), bortezomib (11.6%), cyclosporin (10.1%), cyclophosphamide (10.1%) and tacrolimus (8.7%).

Conclusion:

This study illustrates that TTP has a significant better outcome than other types of TMA. This clinical observation highlights the necessity for investigating other types of TMA prospectively in the future.

508. Does time of day and short-duration high-intensity exercise influence coagulation and fibrinolysis?

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Introduction: Exercise has considerable effects upon haemostasis, with transient increases in blood coagulation dependent upon exercise duration, intensity, and the time of day exercise is completed. The aims of this study were to investigate, 1) acute changes in coagulation activation following short-duration high-intensity exercise across the day in well-trained male cyclists, and 2) whether time of day affected pre-exercise markers of haemostasis.

Methods: Sixteen well-trained male cyclists (VO_{2max} : 59.2±7.2 ml.kg⁻¹.min⁻¹) completed a 4km cycling time trial (TT) on five separate occasions at 0830, 1130, 1430, 1730 and 2030 h. Trials were completed in a randomised order with each TT separated by a minimum of two, and a maximum of seven days. Venous blood samples were obtained pre- and post-exercise, with plasma levels of tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombin anti-thrombin complexes (TAT) and D-Dimer determined.

Results: A 4km TT significantly increased median plasma concentrations of TF, TFPI, TAT complexes and D-Dimer at 0830, 1130, 1430, 1730 and 2030 h (**Table 1**). Furthermore, a pre-TT time of day effect was observed for TF ($p=.004$), with TF greater at 0830 (5.07 (2.08-71.68pg/mL)) when compared to 1730 h (4.01 (0.00-50.43pg/mL)) ($p=.007$).

Table 1. TF, TFPI, TAT and D-Dimer measured pre- and post-exercise at 0830, 1130, 1430, 1730 and 2030 h. Data are presented as median and range.

	0830	1130	1430	1730	2030
TF (pg/mL)	Pre: 5.1 (2.1-71.7) Post: 6.8 (2.4-74.3) $p=0.0001$	Pre: 4.6 (2.0-64.1) Post: 5.8 (2.2-64.8) $p=0.0001$	Pre: 4.2 (1.7-48.0) Post: 5.9 (2.1-48.3) $p=0.0001$	Pre: 4.0 (0.0-50.4) Post: 5.1 (1.9-54.3) $p=0.0002$	Pre: 4.2 (0.0-62.5) Post: 4.8 (0.0-67.9) $p=0.0002$
TFPI (ng/mL)	Pre: 38.5 (55.0-72.5) Post: 46.0 (11.5-94.1) $p=0.0001$	Pre: 30.6 (39.5-70.2) Post: 54.0 (95.7-11.5) $p=0.0001$	Pre: 33.6 (0.4-65.6) Post: 40.6 (10.1-21.7) $p=0.0001$	Pre: 35.3 (45.4-66.7) Post: 50.0 (52.1-11.5) $p=0.0006$	Pre: 41.3 (0.0-73.6) Post: 50.5 (10.5-17.6) $p=0.0001$
TAT (ng/mL)	Pre: 1.7 (1.0-3.0) Post: 3.0 (0.2-4.1) $p=0.0001$	Pre: 2.1 (0.4-3.7) Post: 2.4 (1.4-4.0) $p=0.0012$	Pre: 1.3 (0.5-4.3) Post: 2.4 (1.2-3.9) $p=0.002$	Pre: 1.9 (0.5-3.5) Post: 2.9 (0.5-4.6) $p=0.0001$	Pre: 1.4 (0.5-4.3) Post: 2.2 (1.3-4.4) $p=0.0002$
D-Dimer (ng/mL)	Pre: 73.1 (0.0-2250) Post: 83.3 (0.0-2653) $p=0.0001$	Pre: 52.0 (0.0-1175) Post: 137.3 (1.9-1289) $p=0.0001$	Pre: 47.5 (0.0-1027) Post: 128.6 (0.0-1913) $p=0.001$	Pre: 29.7 (0.0-1202) Post: 123.1 (0.0-2666) $p=0.0001$	Pre: 64.2 (0.0-1434) Post: 121.1 (0.0-593) $p=0.0003$

Conclusion: Regardless of the time of day, a short-duration high-intensity bout of exercise results in acute activation of both coagulation and fibrinolysis. Furthermore, a circadian rhythm was present within the pre-exercise marker of TF, but not within TAT, TFPI and D-Dimer, suggesting caution should be applied when completing short-duration high-intensity exercise between 0830-1130 h.

509. Does wearing compression socks during a marathon influence coagulation and fibrinolytic activation?

Zadow E¹, Adams M^{1,2}, Wu S^{1,3}, Kitic C¹, Singh I⁵, Kundur A⁵, Bost N⁶, Johnston A^{5,6}, Bulmer A⁵, Crilly J^{5,6}, Halson S⁴, Fell J¹

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Introduction: Compression socks are widely used within clinical settings for the prevention of lower extremity clinical pathologies. More recently, their use has widened through the sporting industry due to their proposed enhancement of exercise performance and recovery. The use of compression socks on haemostatic activation during endurance exercise however, has yet to be extensively investigated. The aim was to investigate the effect of compression socks on exercise-induced activation of coagulation and fibrinolysis following a marathon run.

Methods: Forty-three males (mean±SD: age: 46.7±10.3y) and 24 females (age: 40.0±11.0y), were allocated to either a compression (SOCK; n=34) or a control (CONTROL; n=33) group to complete a 42.2 km marathon. Venous blood samples were obtained 24 h prior to (PRE), and immediately post-marathon (POST), and were analysed for plasma levels of thrombin anti-thrombin complex (TAT), tissue factor (TF), tissue factor pathway inhibitor (TFPI) and D-Dimer.

Results: Marathon running significantly increased plasma concentrations of TF and D-Dimer in both the CONTROL and SOCK groups, whereas no significant increases were observed in TAT or TFPI (**Table 1**). The magnitude of change (PRE-POST) for D-Dimer was significantly greater in the CONTROL group, when compared to SOCK (p=0.008), but there were no significant differences for TAT, TF or TFPI (p>0.05) (**Table 1**).

Table 1. TAT, TF, TFPI and D-Dimer measured pre- and post-marathon, with the magnitude of change between conditions. Data are presented as median and range.

	CONTROL (n=33)	SOCK (n=34)	Δ CONTROL vs SOCK
TAT (ng/mL)	Pre: 3.19 (0.16-6.28) Post: 3.09 (0.56-10.23) p=0.236	Pre: 2.47 (0.34-7.31) Post: 2.48 (0.99-7.78) p=0.400	CON: 0.44 (-3.63-6.18) SOCK: 0.36 (-2.69-3.66) p=0.739
TF (pg/mL)	Pre: 3.86 (0.0-17.52) Post: 7.19 (2.16-36.25) p<0.001*	Pre: 5.06 (0.13-14.22) Post: 5.84 (0.84-17.12) p=0.046*	CON: 3.04 (-5.01-32.4) SOCK: 1.19 (-7.47-9.11) p=0.068
TFPI (ng/mL)	Pre: 24.85 (0.29-77.33) Post: 22.85 (0.28-88.53) p=0.840	Pre: 17.90 (0.50-48.50) Post: 17.33 (0.41-72.63) p=0.490	CON: 0.74 (-31.32-58.89) SOCK: 1.46 (-39.28-52.18) p=0.430
D-Dimer (ng/mL)	Pre: 5.22 (0.0-24.14) Post: 33.73 (5.27-87.95) p<0.001*	Pre: 5.50 (0.05-33.32) Post: 16.86 (1.02-78.24) p=0.001*	CON: 25.48 (0.95-73.24) SOCK: 9.02 (-0.34-60.75) p=0.008*

Conclusion: Whilst activation of coagulation and fibrinolysis was apparent in runners assigned to both the CONTROL and SOCK groups, our results suggest overall coagulation and fibrinolytic activation tended to be lower within the SOCK group. Therefore, compression socks may reduce exercise-associated haemostatic activation when completing prolonged strenuous exercise.

510. Uncommon presentations of plasma cell dyscrasia's

Faiman B

The spectrum of plasma cell dyscrasias (PCDs) is vast, compared to other disease states, yet the incidence worldwide is generally low compared to heart disease, hypertension and even other common cancers. Still it remains that disorders of the plasma cell lineage encompasses a number of pre-malignant to malignant conditions of varying severity which clinicians should be aware of. Disorders such as monoclonal gammopathy of undetermined significance (MGUS) may be seemingly benign yet harbour neuropathies which can impair ones' quality of life. More common PCDs such as multiple myeloma (MM), Waldenstroms Macroglobulinemia (WM), light chain immunoglobulin (AL) amyloidosis have distinct algorithms, but appropriate diagnosis and management of rare presentations and unique subtypes can be difficult for the clinician. Thus, this presentation will provide updated diagnostic overview of PCDs, and use a case-based approach to describe unusual and challenging presentations of immunoglobulin light chain amyloidosis, MM, extra-medullary and central nervous system (CNS) disease, osteosclerotic myeloma (POEMS) plasma cell leukemia (PCL).

511. Infection matters in myeloma...especially in the elderly

Joyce T

With Australia witnessing one of the worst flu seasons since the pandemic of H1NI in 2009, it is timely to discuss the impact of infection on our most vulnerable, the immunocompromised and the elderly. A diagnosis of myeloma affects both these patient groups. The median age of diagnosis is 70 years. Increasing age is associated with the decline in physiological reserve and rising co-morbidities, predisposing these groups to increased risk of infection. More importantly, age related immune dysfunction, defined as immunosenescence, is characterised by a decline in the function of the immune system. Not surprisingly, the most common cause of morbidity and early mortality in myeloma patients is infection. It is estimated that 45% of early deaths are due to infection. The peculiarities of aging in relation to infection make management more challenging. These peculiarities include altered epidemiology, atypical clinical presentations and difficulty in obtaining high quality samples for diagnosis. Layer on this a diagnosis of myeloma and a state of immune paresis with deficits throughout the immune system. Not uncommonly patients with a new diagnosis of myeloma will present with life threatening severe bacterial infections because of deficits in the innate immune system. Highest risk periods of infection are associated with greatest burden of disease, namely at early and refractory phases of the disease. Added to this is the milieu of treatments currently available to treat myeloma and the specific impact of each of these on immune function. This paper will discuss the unique and specific infection risks in the older myeloma patient, as well as preventative strategies, including vaccination and anti-infective prophylaxis recommendations. The world is aging with the percentage of people >65 years growing at an unprecedented rate and expected to reach 1.5 billion by 2050 or 16.5% of the global population. This growth will see an increased incidence of cancer, especially myeloma. The interface between haematology , infectious diseases specialty, and gerontology will need to be further developed to provide the specialist care required by this highly complex patient group.

512. How does myeloma impact health related quality of life (HRQoL): The patients perspective

King T

Multiple myeloma (MM) is chronic, debilitating and incurable cancer. The primary goals of treatment are controlling the underlying disease, prolonging survival and maximising health related quality of life (HRQoL) (1). Although new therapeutic modalities have improved length of survival, it has come at some considerable cost to the patient. The burden of MM related symptoms, treatment related toxicities and financial and psychological effects, contribute to increased morbidity and mortality of MM patients and adversely impact HRQoL. A defining feature of HRQoL is that it is a subjective phenomenon so the patients' assessment is preferred over that of a nurse or doctor. The term patient reported outcome (PRO) is "any report of the status of a patients' health condition that comes directly from the patient, without interpretation of the patients' response by a clinician or anyone else" (2 p2) and are considered the gold standard for assessing matters relating to HRQoL. Psychometrically sound instruments that enable clinicians to assess PRO are referred to as PRO measures (PROM).

This presentation will describe what we know about how MM impacts HRQoL; the PROM more commonly used in MM research and clinical practice and propose how nurses can utilise PROM in routine practice to better understand an individual patients' needs, impact of an intervention, and plan patient-centred supportive care (3).

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513. Oncology/Haematology 24 Hour Triage Rapid Assessment and Access Tool Kit - Aussie Style Nationally!

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Introduction:

Promotion, introduction and implementation of a triage tool for patients undergoing antineoplastic therapy experiencing adverse events to be hosted by a nationally recognised platform.

Aim:

- Improve quality and safety
- Standardise care delivery
- Provide an assessment tool/process
- Provide competency training
- Introduce Activity Based Funding
- Provide SMART goal outcomes – specific, measurable, action oriented, realistic/relevant and time bound

Method:

Adoption of the United Kingdom Oncology Nursing Society 24 hour Rapid Assessment and Access Tool Kit copyright program. A working party comprised of consultant haematologists, oncologists, senior nursing clinicians and pharmacy leads have reviewed the tool and requested permission to alter language and practice points ensuring relevancy for Australian patients and clinicians. The tool kit comprises of an alert card, tool kit manual, log sheet, assessment tool and competency framework. The assessment tool prompts the clinician with appropriate questions in order to gain relevant information from the patient to enable fast and efficacious assessment based on the WHO toxicity grading and The NCI Common Terminology Criteria for Adverse Events.

Level of urgency for presenting symptoms are graded utilising a traffic light guide;

- red - patient strongly encouraged to present to hospital immediately,
- two or more amber toxicities are escalated to red,
- single amber toxicities are followed up in 24 hours with patient instructed to call back should condition deteriorate,
- green - instructed to call back if problems continue or condition deteriorates.

Result:

The working party are finalising the Australian version of the tool. Two Australian organisations have implemented this tool with much success. Walk-ins, re-presentations have reduced. Patients report an added level of confidence in their care.

Conclusion:

The Australian version of the **Oncology/Haematology 24 Hour Triage Rapid Assessment and Access Tool Kit** will be available in 2018.

514. Identifying risk of deteriorating and dying in people with a haematological malignancy

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Aim:

To identify the key clinical indicators that signal a person with a haematological malignancy is at risk of deterioration and dying and may benefit from proactive palliative care planning or transitioning to a palliative approach.

Method:

A mixed-method three-step modified Delphi approach was utilised to gather opinions from an international panel of expert clinicians (n=31) on the key clinical indicators that signal risk of deteriorating and dying. Consensus was achieved if 70% of responses fell within two points on a seven-point Likert-type scale. A retrospective case-control design was used to explore the presence of clinical indicators identified in the Delphi in deceased (n=120) and living (n=240) patients who were admitted to the Royal Brisbane and Women's Hospital.

Results:

Eleven clinical indicators achieved consensus in the Delphi as being associated with deteriorating and dying for people with a haematological malignancy. However, in logistic regression modelling in the case-control study, five of these indicators were independently predictive of mortality in the final three months of life including treatment limitations (Odds Ratio 7.86, 95% Confidence Interval 3.53-17.49, $p<0.001$), declining performance status (OR 7.15, 95% CI 3.28-15.60, $p<0.001$), persistent bacterial or viral infections (OR 6.07, 95% CI 2.55 - 14.46, $p<0.001$), relapsed, refractory or persistent disease (OR 3.75, 95% CI 1.75 - 8.04, $p=0.001$) and multi-morbidity (OR 2.99, 95% CI 1.32-6.78, $p=0.009$).

Conclusion:

A combination of objective and subjective clinician-assessed indicators that are contextually relevant to the nature of haematological malignancies were identified as markers of risk by a panel of experts, and via a case-control study. This study has provided valuable preliminary findings on the topic however, more research is required to test these indicators in the clinical setting. This work has the potential to help provide patients with the best death possible.

No conflict of interest to disclose.

515. Best Practice Guidance for Nurses Managing Patients with Paroxysmal Nocturnal Haemoglobinuria Across the Life Spectrum.

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Background:

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disease characterized by chronic, complement mediated intravascular haemolysis. Nurses play a critical role in the holistic management of PNH. Due to the rarity of the disease, experience and knowledge in managing patients with PNH is lacking for many nurses, and there is currently no literature specific to the nurse care setting published to date.

Aims:

To address different issues and aspects of PNH that commonly arise across the different stages of a patient's life, and provide specialised guidance for nurses.

Methods:

This is a collaboration of real-world experience and expert opinion by two world-leading PNH nurse specialists, and is supported by evidence based on current literature. Common issues are discussed in relation to different life stages; 1) teenagers and adolescents 12-20 years, 2) early adults 20-40 years, 3) middle age adults 40-60 years, 4) retirees >60 years, and 5) all life stages, with the intent to prompt and raise discussions that are relevant to each patient.

Discussion:

The most commonly identified issues for each life stage discussed are 1) Behavioural and psychological issues, acceptance of their condition, and adherence to prophylactic antibiotics for teenage and adolescent patients. 2) Contraceptive methods, reproductive concerns, and pregnancy planning and management for early adults. 3) Alternative/holistic therapies and age related comorbidities for middle aged adults. 4) Home care services, cannulation and central device issues and when to stop eculizumab/start palliative care for the retiree age group. 5) Managing travel and preserving vein integrity for all age groups.

Summary/Conclusion:

Nurses play a vital role in managing patients with PNH across their life course. By identifying potential issues and providing best practice guidance on matters that may arise during a patient's life journey, nurses can be better educated and equipped to provide the best care for their patients.

516. The implementation of an information management system and electronic Chemotherapy prescribing in a Cancer Centre

Steele A¹

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This presentation will outline the implementation of the Elekta MOSAIQ information management system in a busy tertiary hospital cancer centre, for haematology, medical oncology, radiation oncology and allied health.

Aim:

The aim of the project was to implement an information management system in the Cancer Centre of Sir Charles Gairdner Hospital in Perth. The management system included electronic medical records, an internal messaging system for referrals from clinic areas and inpatient teams, as well as the facility to e-prescribe chemotherapy orders and administer chemotherapy with electronic records.

Method:

A team was assembled to coordinate and facilitate the project and configure the software to the specific needs of each discipline. The MOSAIQ project team was multi-disciplinary with input from medical specialists, nursing, clerical, radiotherapy and clinical trials. As it involved teams from haematology, medical oncology and radiation oncology processes were aligned as much as possible to ensure a streamlined approach.

Result:

The project was implemented step-wise from December 2015 with the final phase of go-live in November 2016. Unforeseen technology issues with the network infrastructure slowed down the system but the processes put in place ensured that treatment of patients in Haematology was not disrupted. Patients now receive their treatment following a care plan implemented electronically and all patients' records and assessments are available to all users improving the communication and written records of the patients seen. There are facilities to audit treatments given and patients seen and even home treatments can be coordinated easier with additions to the scheduling process. Instant referrals are created from clinic to treatment areas.

Conclusion:

The implementation of e-prescribing and integration of records and assessments has successfully improved communication and documentation of the patients' experiences in the Cancer Centre.

517. Bedside to bench and back again. Using a laboratory model to answer a clinical question.

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Background:

Recommendations prescribe daily intravenous administration set (IVAS) replacement for parenteral nutrition (PN) comprising intravenous fat emulsions (IVFE). This may contribute to central-line associated bloodstream infections (CLABSIs).

Methods:

Pre-clinical laboratory experiments and model development to simulate PN administration after contamination from health care workers hands. This study observed the growth of micro-organisms known to cause CLABSIs in patients receiving PN using a variety of PN solutions and other intravenous fluids, and developed an IVAS model to investigate the effect of hang time of up to seven days for IVAS in PN administration containing IVFE on the risk of fluid colonisation.

Result:

Each micro-organism grew at different rates and was affected by the solution. In the static experiments, growth was generally supported in IVFE and all-in-one PN, but suppressed in 50% glucose. There was consistency in the growth patterns over time of *Staphylococcus epidermidis*, *S. aureus* and *Candida albicans* in IVFE, all-in-one PN and 0.9% sodium chloride when results were compared between the static experiments and the dynamic IVAS model. In the IVAS model *C. albicans* grew exponentially in all-in-one PN and IVFE IVAS after two days. Minimal colonies of *S. epidermidis* and *S. aureus* were observed in the IVFE IVAS.

Conclusion:

There is potential for micro-organisms to grow in all-in-one PN and IVFE and migrate from the fluid bag to the distal needleless connector. Improving aseptic non-touch technique during clinical practice will minimise the potential for micro-organisms to be introduced into a sterile system. Increasing the frequency of IVAS replacement should be examined in a good quality, adequately large, randomised controlled trial.

518. HIV in lymphoma patients and transplantation

Milliken S

St Vincents Hospital

Non-Hodgins' Lymphoma (NHL) has been a recognised complication of HIV/AIDS since the epidemic was first recognised.

Results of treatment were initially very poor due primarily to the patients poor performance status with toxicity due to infection a major problem.

The advent of effective combination antiviral therapy in the mid 1990's changed the paradigm for treatment. The incidence of systemic NHL fell by more than 50% and primary CNS NHL went from a common to rare complication due to HIV positive individuals maintaining better immune function. Patients with lymphoma were much better able to tolerate therapy. As a consequence results of therapy now parallel those for non-HIV infected lymphoma patients. Interestingly the relative frequencies of different types of lymphoma have changed with Burkitt-like and Hodgkin lymphoma now relatively more common than DLBCL.

These improvements in maintenance of immune function have also allowed the safe utilisation of high dose chemotherapy and autologous as well as allogeneic haemopoietic stem cell transplantation for those who relapse post primary therapy or present with very high risk disease.

Interesting data is also evolving regarding the positive impact of allogeneic transplantation in improving HIV control. A number of strategies to control HIV infection utilising autologous stem cell transplantation are being trialled.

It is hoped that further advances in control of HIV infection and preservation of immune function will significantly further reduce the incidence of lymphoma in HIV positive individuals.

519. Source of the Graft: Indications and Applications

Sipavicius J

Royal North Shore Hospital

This presentation will include an overview of the interplay and multifaceted decisions that are considered when choosing a graft source for an eligible adult allogeneic haematopoietic stem cell transplant (HSCT) candidate. Historically the most ideal donor for many haematological malignancies in adults was a Human Leukocyte Antigen (HLA) matched sibling donor using peripheral blood as a stem cell source. However less than 20% of patients will have a HLA matched sibling donor. If an alternative donor(s) is available, considerations in terms of HLA match, as well as donor age, gender and cytomegalovirus (CMV) status need to be considered, within the context of patient disease and risk of relapse, conditioning regime and urgency of transplant. However these decisions are not always straightforward, particularly if several donor options are available, and much of the literature is either conflicting or inconclusive. No widespread internationally agreed selection algorithms are available, leaving choice of graft made by a combination of all the above, as well as what donor option(s) are available, decision by treating physician and experience of transplant centre. Furthermore in recent years there has been an increased worldwide activity of using haploidentical donors, potentially changing the landscape of transplant – with the ability to offer a transplant for almost any prospective transplant recipient, and has become the largest donor source in some countries. A summary of some of the key literature and trends in donor selection will be addressed.

520. Transition of care paed - adult

Twist I

The Children's Hospital, Westmead

The CHW performs 25-35 blood and marrow transplants (BMT) a year, both autologous and allogeneic. These referrals for transplant are for both malignant (60%) and non-malignant disease (40%).

The introduction of a nurse-led service in 2009 allows a BMT follow-up Nurse Practitioner (BMT FU NP) to care for survivors of BMT from 6 months' post-transplant. The main objective of this service is to assess and manage the chronic complex consequences of BMT.

There are currently 220 patients in this service ranging in age from 6 month to adulthood. There are 11 patients who are 16yrs or older and are less than 5 years post-transplant, and there are 60 patients over 18yrs old. We have transitioned less than 20 patients into adult BMT services over the last 10 years.

This paper will discuss the rationale for life long follow-up care in post-BMT patients and the barriers to transition of paediatric survivors into adult services in NSW.

521. Central line failure in allogeneic transplant patients

Haywood P¹, Xie M¹, Stevens K¹, Rivalland A¹, Norwood O¹

¹Royal Melbourne Hospital, Parkville, Australia

Aim:

Long term venous access with a central line (CL) is necessary for allogeneic transplant (alloHSCT) but frequently results in complications, such as infection. Although there is an abundance of published data examining CLs, very little is exclusive to alloHSCT. We aim to elucidate the rate and reasons for CL failure, including infection, in a cohort of alloHSCT patients.

Method:

We retrospectively reviewed medical files for all patients who underwent alloHSCT after August 2016 who had reached day 100 before June 2017 in our institution. Line type, insertion date, reason for removal, alteplase prescription and results of all blood cultures were collected. We applied several definitions of infection to the data, in order to compare with different previously published studies. We used Fisher's exact test to establish statistical significance of categorical variables.

Result:

We reviewed 102 CLs from 54 patients over 8245 line days and 1217 sets of blood cultures. The reasons for removal were; 35 (34%) suspected infection, 5 (5%) mechanical failure, 3 (3%) occlusion, 59 (58%) no longer required, which included 13 (13%) patients that died. On 9 occasions a patient met the definition for catheter related blood stream infection (CRBSI) (1.1 per 1000 catheter days), on 10 occasions central line associated blood stream infection (CLABSI), but on only 4 occasions were both the definitions for CRBSI and CLABSI met. 31 doses of alteplase were prescribed, more likely in peripherally inserted CL (PICC) than in tunnelled Hickman catheters (16.6 per 1000 line days vs 2.5, $p = 0.0001$)

Conclusion:

CL failure in alloHSCT, although frequent, was similar to previously published data. Caution should be taken when attempting to compare alloHSCT with other groups given the difficulty in defining CL infection. Tunnelled catheters probably have fewer complications than PICCs in alloHSCT.

522. Care Down There - Vulvovaginal Chronic Graft Versus Host Disease

Hogg M¹

¹Westmead Hospital, Wentworthville, Australia

Vulvovaginal cGVHD is an important but often unrecognised complication following blood and marrow transplant (BMT). Reported incidence is said to be between 35-50% of all female BMT survivors^{1,2,3} internationally and 49.6% within Australia^{4,5}.

The onset of vulvovaginal cGVHD usually occurs 7-10 months post BMT, but may develop years later. The challenge of providing best care for our female survivors is the insidious nature of onset, with many being asymptomatic. It is frequently associated with oral and/or cutaneous cGVHD and can result in erosions, fissures, ulcerations, scarring and agglutination of the vaginal vault or labia. This has a profound impact, with many experiencing sexual dysfunction, relationship difficulties and a reduction in quality of life.

In 2012, the Long Term Follow Up (LTFU) clinic at Westmead funded a gynaecology clinic to address the issue of vulvovaginal cGVHD as the result of cases that had arisen, one of which resulted in total vaginal occlusion. Informal interviews conducted by the LTFU BMT CNS discovered that female BMT survivors rarely felt comfortable reporting symptoms spontaneously. In addition many in the BMT team did not think it pertinent to ask, or felt uncomfortable broaching the subject.

An educational resource on vulvovaginal cGVHD and process for referral were developed. The educational resource was sent to all female BMT survivors and added to the inpatient discharge information. All female BMT survivors would be routinely referred to the gynaecology clinic on transition to LTFU. The CNS initiated discussion with these patients and reiterated the importance of review and referral, which reduced unease and allowed for conversations to occur.

This presentation is designed to share our local experience of vulvovaginal cGVHD through case studies and highlight the vital importance of early recognition and education to prevent and/or minimise the severe sequelae of vulvovaginal cGVHD.

We must all start talking about care down there
No conflict of interest to declare*

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523. Factors influencing health outcomes during transplantation

Guy A¹

¹*Cdhb, Christchurch, New Zealand*

Aim:

Undergoing bone marrow transplantation creates significant physical, social, psychological, and emotional stress. How the individual copes with these challenges contributes to their overall health outcomes. This presentation will explore reasons behind this, in two different ways. Part one will be an exploration of two comparative patient journeys. Part two consists of an analysis of patient-based survey delving into individual experiences.

A high percentage of the patient's cared for on BMTU fall into the cohort of male, aged thirty to sixty-five, who have had prolonged hospital admissions due to intensive therapy. This is the focal group for this presentation.

Part One:

Patient A spent a majority of his time in protective isolation. He was from out of town and had no local support network. In conjunction with cyclic fevers and sleep deprivation Patient A was on the verge of a 'mental breakdown' resulting in heavy reliance on pharmacological interventions. In comparison Patient B has a young family, large support network and was previously fit and healthy. He has experienced massive physical changes and suffered the loss of a friend, diagnosed at a similar time. Both men are at different development stages and utilised vastly different coping mechanisms.

Part Two:

The patient-based survey will provide a collaborative insight into how this cohort grappled through the physical and mental struggles of their illness and treatment. Focus of the survey will include coping strategies, pharmacological and non-pharmacological interventions and areas for improvement.

Conclusion:

This presentation highlights how the defined cohort copes holistically throughout their patient journey. This review will provide the opportunity to compare the past, present and future BMTU unit. To enable a chance to foster innovative ideas for the new facility currently being built in Christchurch.

524. Half way there: a single adult centre experience with a developing haploidentical transplantation service

Deren S¹

¹*BMT Service, Westmead Hospital, Westmead, Australia*

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for a many haematological diseases. Haploidentical (Haplo) HSCT using a T-cell replete graft offers a suitable treatment option for patients who lack human leucocyte antigen (HLA) matched donors. Many haplo HSCT protocols have been successfully established, with promising clinical outcomes as our understanding of these processes improves.³ Westmead Hospital commenced performing haplo HSCT in 2008, with a total of 68 transplants having been performed to June 2017. Twelve (17.6%) received myeloablative conditioning with busulfan and cyclophosphamide and the remainder received reduced intensity conditioning (RIC) predominantly with Fludarabine, cyclophosphamide and single fraction total body irradiation (TBI) in the outpatient setting, with admission only as clinically indicated.

In haplo HSCT donor searching almost all patients have more than one potential donor.⁴ Early identification of the need for haplo HSCT donor searching, and an awareness of the effects of factors such as donor-specific/recipient-specific antibodies against HLA, donor age, gender, ABO and CMV serostatus aids in ensuring donor searching is both timely and complete. A significant goal of searching and donor selection is identifying the most suitable and eligible donor, as this can have a significant impact on the incidences of graft-versus-host disease, transplant-related mortality (TRM), and disease relapse.⁵

Almost a decade of experience with haplo-HSCT has led to changes in the way we provide planning and coordination of this patient group. Our experiences of haplo HSCT have led to an evolving service, with changes being made to the way donors are searched for and screened, to allow for better patient outcomes, decreasing the risk of TRM and other complications. In collaboration with NSW Transplantation and Immunogenetic Service (TIS), the teams at our referral hospitals and our Haematology service we have been able to ensure that we can provide a service which meets the needs of this growing and complex service.

1

³ Kanakry CG, Fuchs EJ, Luznik L. Modern approaches to HLA-haploidentical blood or marrow transplantation. *Nat Rev Clin Oncol.* 2016; 13(2):132.

⁴ Ciurea SO, Bayraktar UD. “No donor”? Consider a haploidentical transplant. *Blood Rev.* 2015; 29(2):63–70.

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525. Nurses Symposium: Sickness comes on horseback but departs on foot: addressing the financial impact of haematology treatments on patients and their families

White K

Increasing attention is being given by the cancer community as well as by mainstream media and government agencies to the impact of the cost of cancer treatment on patients and families. Studies have identified an escalating cost burden experienced by patients. The majority of this research focuses on patients with solid tumours.

This session brings together presentations which will examine and explore current knowledge of the financial implications of cancer, with an emphasis on haematological cancers, for patients and their families as well as available support for those who are experiencing financial challenges.

Objectives:

- Provide participants with an overview of current research on the topic.
- Examine how health costs and reimbursements are changing.
- Present findings from a study of haematology patients in Sydney on the financial impact of their treatment.
- Increase awareness of services available through Non-Government Organisations to assist patients and their families experiencing financial difficulties.

This is a practical and interactive session which will provide attendees with an opportunity to explore this important topic.

526. Sickness comes on horseback - Panel discussion

Kenny P

Centre for Health Economics Research and Evaluation

Abstract not supplied

527. TBC

Lehane L
Sydney Nursing School, The University of Sydney

Abstract not supplied

528. Panel: Sickness comes on horseback but departs on foot: addressing the financial impact of haematology treatments on patients and their families

Bisen A
Cancer Council NSW

Abstract not supplied

530: Workshop: How to turn a clinical question into a research project

Fry M

Nurses frequently recognise areas of practice that require further development, where current practice is not evidence based. Ideas for developing practice may be discussed but go no further, as often skills or time to progress ideas is challenging and the research path unknown. This workshop aims to introduce you to how to ask a 'researchable question'; develop an idea into a research proposal; select a research design, select techniques and collect appropriate data; and apply for ethics and governance. All clinicians need to build researcher skill and capacity to undertake research or translate evidence. This workshop will offer a practical approach to exploring clinical problems and lead service reform.

531. You can ask that: Sex matters

White K

Cancer Nursing Research Unit (CNRU), Sydney Nursing School, The University of Sydney.

Abstract not supplied

532. Targeting your talent-who needs a specialist haematolo-oncology nurse?

Krishnasamy M

University of Melbourne Centre for Cancer Research

Abstract not supplied

533. The changing treatment paradigm in AML

Arthur C

Abstract not supplied

534. Screening and assessment of the older person with cancer

Berry R

Aim:

To demonstrate how an innovative model of older persons oncology care can be implemented to support effective and appropriate treatment for Older Australians with cancer.

Background:

Ageing is heterogeneous and is a risk factor for many cancers and other chronic health problems. As we age other issues may also influence how each patient functions. Despite international recommendations, formal assessment and identification of care needs of older cancer patients and their carers has not been a standardised practice in Australia. To address this issue, the Princess Alexandra Hospital (PAH) developed a research project comparing the assessment of older cancer patients (with solid tumours) by an Oncologist and a nurse initiated comprehensive geriatric assessment (CGA).

Method:

Patients over the age of 70 years are screened with those identified as vulnerable receiving a full CGA. This formal nursing assessment identifies each patient's strengths and health issues, providing in-depth information individualised to the patient. This information is provided to the relevant treating team to support decision making in consultation with the patient and carers.

Result:

As a result of this project, the service has expanded to include patients with haematological malignancies. In the past 12 months 209 patients have been screened with 121 patients requiring a full CGA nursing assessment.

Conclusion:

The Older Person Oncology CNC works closely within the wider multidisciplinary cancer team to develop a person centred care plan. Identifying non-cancer related issues gives the opportunity to plan appropriate care and provide extra support during treatment and beyond- ensuring care is specific to each patient's needs.

The Older Person Oncology CNC position is a pivotal role in planning and providing cancer care of the older person, while also identifying potential opportunity for research in this specialty.

535. Whose dose is it anyway? The legal, medical and nursing implications relating to delivery of cancer therapy

Taylor S

In recent times, there has been a large amount of media coverage throughout Australia about the chemotherapy under-dosing errors. This coverage has sparked a lot of attention into the quality and safety of cancer care and the importance of implementing and de-implementing evidence into practice.

This session includes a panel of members from different specialities who will discuss their viewpoints from a legal, medical, nursing, management and pharmacy perspective. The aim of the session is to generate discussion amongst the audience about a topic that is very relevant in this current climate.

Objectives:

To discuss:

- Quality and safety in cancer care.
- The importance of quality improvement
- Policies that have been implemented as a result of the cancer care errors
- The challenges with implementing practice
- The approaches that have been developed to standardise practice

536. Improving outcomes in the management of massive transfusion (MT) management in a regional setting

Regester D¹, Wright T¹

¹Latrobe Regional Hospital, Rosanna, Australia

Introduction: Latrobe Regional Hospital (LRH) is the regional health service for Gippsland. Implementation of the massive transfusion protocol (MTP) in 2014 focused attention on the range of obstetric, trauma and surgical massive transfusions managed in our hospital. Routine review of the clinical management of MT identified recognition of MTP triggers and MT activation as areas requiring improvement.

Aim:

- Establish a comprehensive audit of all massive transfusion events at LRH
- Identify areas of divergence of MT management from current protocol
- Use outcomes from the audit to implement a range of clinical and practical changes to MT management supported by a comprehensive education and training program

Method: The National Blood Authority Clinical audit tool¹ was adapted to provide a focussed audit around recognition of MTP triggers, MT activation and initiation.

From November 2016 to June 2017 all MT meeting the protocol criteria were audited. Each MT was assessed for appropriate activation, stand down, initial pathology collection, ongoing pathology collection and clinical measures.

Interventions included development of clinical support tools & a comprehensive multidisciplinary education and simulation program across key specialty areas.

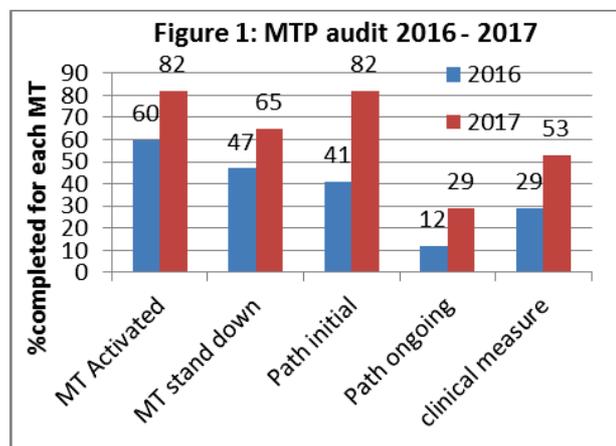
Audit data was compared before and after implemented practice changes.

Results: From the period November 2016 to June 2017, 34 clinical events met the criteria for a MT

Figure 1 summarises pre and post audit results following the quality improvement program. Improvements were recorded in all audited areas between 17 to 40%

Staff feedback of simulation events concluded increased confidence in activation and management of MT

The improved clinical tools have been adapted with respect to Theatre/Anaesthetic training, Advanced Life Support and PRactical Obstetric Multi Professional Training (PROMPT) workshops



Conclusion: Use of a simple regular audit tool for all MT events provided guidance for appropriate intervention to improve MT management. Directed clinical and education interventions resulted in measurable and consistent improvements. Staff satisfaction and competency in a challenging clinical area subsequently improved. A quality assurance program focussing on improving fundamental aspects MT management will allow ongoing improvements including appropriate use of blood products in MT and from this participation in the Massive Transfusion Registry.

¹<https://www.blood.gov.au/massive-transfusion-protocol-mtp-clinical-audit-tool>

537. Time out for blood administration in the emergency department

Saddington E¹, Walker C¹, Murdoch K¹, Symonds M¹, Charlton S¹, Pickles L¹, **Akers C¹**
¹*Alfred Health, Prahran, Australia*

Aim:

Improve the process for blood administration in the emergency department (ED).

The ED at Alfred Health had 4 incidents over a 2 year period where patients received blood intended for another patient.

Method:

Development of multidisciplinary working group comprised of clinical nurses, management staff, (nursing and medical), transfusion nurse and educators.

The group wanted to understand why the checking process is not followed correctly and devise tools to improve this.

Focus groups met to discuss issues and processes, where they saw the problems and contributing factors.

A thematic gap analysis helped to identify barriers in the ED to safe practice.

Issues included time and space to perform checks, in massive transfusion events the person administering was not one of the checkers, interruptions, confusion around roles and communication.

The group then determined ways to address the main themes found.

Result:

As a result there were two areas of work. The first was around communication with the laboratory, using ISBAR for all communications and ensuring senior staff awareness of transfusions occurring.

The second was to provide a safe place to perform the blood checking by providing transfusion trollies with high visibility vests for staff when performing the checks. These vests highlights the staff member is not to be interrupted and this is respected by all staff.

Since the introduction there have been no further serious incidents relating to incorrect product to patient. The staff continue to use the process as demonstrated in both internal and external audits.

Conclusion:

Although this process took considerable work, it has been very successful in reducing the number of serious incidents occurring in ED. Promotion within the hospital has encouraged others to consider use of trollies and vests for transfusion. We will continue to work with these areas to assist in implementing this simple yet effective tool.

538. MegBio: Accessing tissue from past leukaemia patients to guide the future - is it ethical?

Wylie J¹, Petrasich M², Hunter S³, Chan G², Teague L³, Kaley M^{1,2}

¹Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand, ²Haematology Laboratory, LabPLUS, Auckland City Hospital, Auckland, New Zealand, ³Blood and Cancer Centre, Starship Children's Hospital, Auckland, New Zealand

Aim:

MegBio is a multi-modal project aiming to characterise the disease mechanisms underlying megakaryocytic cancers and identify new therapeutic targets. Acute megakaryoblastic leukaemia (AMKL) is rare, though children with Down syndrome (DS) are 500 times more vulnerable. Beside DS, AMKL accounts for 7-10% of paediatric AML and 1-2% of adult AML, where it frequently arises secondary to myeloproliferative neoplasms (MPN). The adult median survival is only 23 weeks. DS children fare better but tolerate chemotherapy poorly. DS newborns are also prone to transient abnormal myeloproliferation (TAM), which in 20-30% develops into AMKL. The reversible nature of TAM could provide important molecular clues to AMKL pathways and treatment approaches.

Methods:

Because AMKL is so rare, we applied for ethical approval to access a subset of historical samples from past AMKL patients, archived over a 20-year period for quality and development purposes. Our discoveries from this valuable resource will underpin more comprehensive profiling in a prospective study arm enrolling adult and paediatric patients with acute and chronic megakaryocytic cancers including MPN and individuals with DS.

To support our retrospective sample application, we undertook wide consultation with patient consumers, Maori, and a medical ethicist. The ethics committee approved use of the tissue without consent from next of kin based on our inability to enrol sufficient new patients, fairness

Disease type	Children	Children alive	Adults	Adults alive	Total alive	Unknown status	Total patients
1 ^o AMKL	7	5	1	1	6	1	9
2 ^o AMKL		0	8	0	0	1	9
Trisomy 21, TAM only	7	6	-	-	6	0	7
Trisomy 21, AMKL	7	6	0	-	6	0	7
Total	21	17	9	1	18	2	32

and potential to distress patients' families. This meets Health Research Council ethical guidelines for research use of non-consented human material.

Results and Conclusion:

Medical record searches now reveal, that 18/32 or 56% of patients in this cohort remain alive (two unknown), the majority surviving childhood AMKL or TAM (Table 1). This raises new questions around how to best inform and gain the consent of this group for use of their samples. We are working with paediatric colleagues and the Ethics Committee to revise our approaches.

Table 1. Status of patients in retrospective cohort (children/adults = age-group at diagnosis)

539. Large volume leukapheresis versus standard volume leukapheresis – Western Australian experience.

Eisler M¹

¹*Wa Health, Murdoch, Australia*

The apheresis centre at Fiona Stanley Hospital (FSH) in Perth performs haematopoietic progenitor cell collections via apheresis (HPC(A)) from autologous and allogeneic (related and unrelated) donors.

A formula is used to accurately calculate the blood volume required to process at apheresis to achieve target cell collect:

CE = Collection Efficiency factor, usually 0.4 or 0.5.

Aim:

Applying the formula above often results in donors undergoing Large Volume Leukapheresis (LVL), which is defined at FSH as processing more than 3 donor's Total Blood Volumes (TBV) or more than 15 litres of blood.

The aim of this audit was to determine whether patients who undergo LVL are at increased risk of citrate toxicities and adverse events during apheresis, and the impact of lowered electrolyte levels and platelets after the HPC(A) procedure.

Method:

113 donors were included in an audit at FSH from January 2016 to May 2017.

The recorded parameters were: autologous/allogeneic donor, citrate toxicity that occurred, Blood Volume processed, essential electrolyte and platelet levels pre and post procedure. The data was assessed for possible correlations.

Result:

Donors experience citrate toxicities in both groups (LVL and non-LVL) however this incidence is higher in the LVL group. 68% of donors who underwent LVL suffered citrate toxicity, as opposed to 32% of those who underwent standard volume leukapheresis. Donors in the LVL group are more likely to have subtherapeutic electrolyte levels post HPC(A), and reductions in platelet counts.

Conclusion:

LVL is a safe and cost effective practice, and is convenient for donors. However, collection centres need to be aware of its potential impact on donors and establish their own guidelines on the management of citrate toxicities and electrolyte imbalances post HPC(A).

540. Building, understanding and measuring advanced practice roles: the state of play and future hopes

Fry M

The implementation of specialist endorsed Haematology Nurse Practitioners (NP) is a desirable and necessary intervention if we are to work towards meeting service demand, equity and quality of care. In Australia, the NP's role is built upon the scope of practice of the registered nurse and complies with the national competency standards, Code of Ethics, and the Code of professional conduct as outlined by the Nursing and Midwifery Board of Australia. The NP is defined as an advanced, clinically experienced registered nurse, who holds an academic postgraduate degree (Masters Level).

Internationally, there are more than 95 NP roles some of which include: haematology, oncology, intensive care, emergency, surgical; cardiology, neurology and general medicine services. Within these services NPs are managing acute and chronic patient conditions within in-patient and/or outpatient settings. The NP role has been well established in the USA (1960s), UK (1980s), Canada (2000) and to a lesser extent Australia (1995) and New Zealand (2000). In the USA, the AANPs identified that there were 234,000 licensed NPs with 23,000 educated in 2015-2016.

In Australia, the scope of practice, independence and autonomy of NPs is significantly less than international evidence. Yet no appreciable difference has been found between NPs and doctors in health outcomes for patient management or resource utilisation. However, in the majority of studies patient satisfaction scores were greater and NPs tended to be more reliable in following best practice guidelines and medical record documentation.

Building and designing Haematology NP roles require considered thought, marketing, vision and creativity and will usually start with a business case. However, stakeholder engagement and identification of service need will ensure a better fit between patient and organisational need and NP responsibilities. NPs need to be resilient and courageous to give shape to innovation and redefine the landscape of haematology services. Consequently, how to measure NP outcomes needs to be considered early and as an ongoing process.

To date, while the quality difference in patient outcomes for is mixed, studies have demonstrated NPs achieve patient and hospital savings. Looking to the future NPs need to measure the economic impact of their model of care. Further, there is little evidence on which to gauge the response of consumers toward NP care or the impact of competition between Physician and non-Physician groups within Australia. Internationally, NPs lead acute and chronic healthcare service models that are sustainable, acceptable, efficient and affordable. This presentation will discuss building, understanding and measuring advanced practice roles; looking forward and beyond to new ways of thinking.

541. Targeting your talent-who needs a specialist haematolo-oncology nurse?

Krishnasamy M

University of Melbourne Centre for Cancer Research

Abstract not supplied

542. Embedding advanced practice nurse roles into a haematology service

Faiman B

More than ever, hematology and oncology practices rely on advanced practice nurses (APNs) to fill the unmet needs created by a growing patient base and a shortage of qualified physicians. The benefits of APNs have been well described in the United States and other areas of the world; yet, barriers exist which prevent APNs from being integrated effectively into ones' practice. As one of the first APNs in hematology and medical oncology at a leading hospital in the United States, I have worked to build an independent yet collaborative practice. This presentation will highlight important factors for the APN to consider when building a practice in haematology, strategies for measuring patient satisfaction, and data for clinical outcomes. Clinical pearls for team building, the importance of continuing education, and methods to obtain quality scores will also be shared for those who intend to set up positions locally.

546. Hypereosinophilia ALL – rare and unusual

Maddock K¹

¹*Westmead Hospital, Westmead, Australia*

This presentation introduces the rare and unusual case of JF a 19year old who presented to a satellite hospital with a sore throat, vomiting for 4 days and dehydration. Routine blood pathology was performed in the emergency department and urgent haematology lab results showed haemoglobin was 127g/L, WCC was 224×10^9 /L with an eosinophil count of 193×10^9 /L and platelet count 200×10^9 /L.

Hypereosinophilia leads to several possible diagnostic possibilities, asthma, allergies or auto immune disorders, invasive parasites or drug induced hyper-sensitivities, haematological disorders such as Chronic Myeloid Leukaemia, Hodgkin Disease, Myeloproliferative Neoplasm, pernicious anaemia or a lymphoid blast crises of Chronic Myeloid Leukaemia. It is important to diagnose the cause and swiftly commence treatment, directed at the disorder stimulating the eosinophil production, as end-organ damage occurs with persistent severe hypereosinophilia. Common organs effected include the lung, skin and gastrointestinal tract. Cardiac and nervous system damage is of more concern and potentially life-threatening.

The young woman required urgent transfer to a major metropolitan hospital. Hypereosinophilia at first clinical presentation, the difficulty diagnosing the disease driving the hyper eosinophilia and the supportive care required before and during initial treatment is intensive. A thrombus in the left brachiocephalic vein extending into the left subclavian vein, troponin counts of greater than 5,000ng/L, tachycardia >150 at rest and an echocardiogram showing regional wall motion abnormality with pericardial fluid were observed.

A diagnosis of an Acute Lymphoblastic Leukaemia (ALL) clone on bone marrow flow cytometry analysis was made. There have been approximately 48 cases reported in the literature of hypereosinophilia ALL since the 1970's. In this case the disease failed to respond to steroids and the eosinophil count continued to rise requiring urgent leukapheresis. With leukapheresis and the commencement of BFM 2000 ALL protocol the blood levels improved as did the cardiac and cognitive function of this bright young woman, who has now commenced induction 1B and remains MRD positive.

"No conflict of interest to disclose".

547. Telephone encounters: responding to patient reported change in clinical status – an innovative approach

Lawrence C¹

¹*Wa Cancer & Palliative Care Network, Perth, Australia*

Background:

The Australian National Safety and Quality Health Service (NSQHS) Standards provide a governance framework for safety and quality in health care. Standard 9 addresses recognition and response to clinical deterioration in the acute healthcare setting. The Western Australia Cancer & Palliative Care Network (WACPCN) Cancer Nurse Coordinators (CNC) provide a central point of contact for cancer patients and their caregivers, and provide holistic needs assessments throughout the patient's journey. Significant work is conducted over the telephone and may involve assessment and management of patient reported change in clinical status.

It is not possible to accurately determine clinical deterioration over the telephone. However it is possible to identify patient reported change in clinical status and provide a safe escalation of care response.

Aim:

The objective of this project was to enhance patient safety and quality of care by addressing the Standard 9 intent for escalating care in response to patient reported change in clinical status over the telephone.

Method:

A nurse-led working party was convened to develop a policy addressing relevant criteria of Standard 9. A telephone encounter algorithm was designed for incorporation into the policy, and an audit undertaken to identify current gaps in CNC reportable telephone encounter (RTE) form documentation.

Result:

The policy and algorithm have been developed for use and provide a standardised approach to determine and facilitate the escalation of care process in the non-acute setting. Audit findings highlighted inconsistencies in the use of the RTE form.

Conclusion:

This innovative change in practice enhances patient safety and meets the intent of NSQHS Standard 9. The algorithm is a valuable resource in providing a standardised approach to manage patient reported change in clinical status and escalation of care over the telephone. Modification of the RTE form will address the need for improved documentation.

548. When do peripheral blood cultures add value in febrile haematology patients with a central line?

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Aim:

Blood cultures (BC) taken peripherally during a febrile episode are thought to add value in identifying bacterial and fungal septicaemia in patients with a central line. However, very limited published data is focused on neutropenic/immunosuppressed patient groups. We sought to investigate how often peripheral blood cultures added value to diagnosing the source of infection or identifying species of septicaemia in patients with a central line.

Method:

We retrospectively reviewed medical files for all BCs taken in our 32-bed acute leukaemia and allogeneic bone marrow transplant unit between April 2016 and May 2017. Site of BC draw, BC result and time to positivity were collected. A central BC that was positive two hours sooner than a paired peripheral BC was considered to have a relevant differential time to positivity (DTP).

Result:

We reviewed 4227 sets of BCs from 363 patients. There were 989 distinct paired episodes that included at least one peripheral and one central BC, of which, 122 (12.3%) included at least one positive BC. On 62 (6.4%) occasions there was a discordance between the peripheral BC result and one or more central BC result. On 7 (0.7%) occasions the peripheral BC was positive, but all central BC were negative. On 5 (0.5%) occasions the peripheral BC and at least one of the central BC were positive and a recorded DTP was greater than 2 hours. On average, it took 82 sets of peripheral BC to be taken, for one of them to demonstrate a result that was not found in a central BC.

Conclusion:

Although often considered a minimally invasive procedure, peripheral BCs are a potentially overvalued diagnostic procedure in unwell acute leukaemia and allogeneic transplant patients who have a functioning central line. These results differ markedly from published reports of similar studies in general hospital patients

549. Plerixafor use in poor mobilisers: How low can you go?

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There is no doubt that Plerixafor has been a game changer in enabling poor mobilisers to collect adequate PBSC and proceed to ASCT with successful and sustained engraftment. Since FDA approval in 2008, efforts have focused on how to optimise its use in order to minimise costs and maximise outcomes.

Our experience commenced in late 2008—using the recommended rescue strategy of a second mobilisation with GCSF + Plerixafor. Of this 17 patient cohort, 11 (65%) achieved an apheresis CD34 dose of $\geq 2 \times 10^6/\text{kg}$. 10 of the 11 (90%) underwent ASCT.

For the past 5 years our practice has utilised the more efficient pre-emptive strategy—incorporating the algorithm of WCC >5 and PB CD34 <10 as a trigger for use. ChemoGCSF is the preferred mobilisation regimen. Plerixafor is given at 5pm in the outpatient unit to negate the need for overnight admission.

Yet to be clarified is the level at which the pre Plerixafor PB CD34 is unlikely to result in collection of a transplantable CD34 dose. Our aim is to determine a reasonable cut off for patients with very low circulating CD34 counts and update our algorithm with clear parameters for selecting those who may benefit most. This single centre retrospective study presents the outcomes of 44 patients given Plerixafor since Sept 2012.

40/44 (90%) of patients with pre Plerixafor PB CD34 counts ranging from 2-10 uL were successfully mobilised with enough cells to allow Autologous transplant. Of the 4 mobilisation failures, pre Plerixafor PB CD34 counts were <2 . Variables such as age, white cell count, diagnosis and pretreatment will be included in this study. Despite our limited cohort, we advocate the use of Plerixafor in patients with very low PB CD34 levels.

550. Stem Cell Collection post High Dose Melphalan and Autologous Bone Marrow Transplantation

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Aim:

The addition of salvage treatment for Relapsed Multiple Myeloma (RMM) with Autologous Bone Marrow Transplantation (ABMT) can provide progression-free and overall survival benefit. Furthermore, Salvage treatment with ABMT for Multiple Myeloma (MM) is associated with a low risk of mortality. The aim is to evaluate whether PBSCC can be safely and efficiently collected after previous treatment of High Dose (HD) Melphalan and ABMT.

Background and Results:

Nepean Hospital, offers salvage treatment for suitable patients with RMM. Salvage ABMT has led to the change in practice where the aim of stem cell collection is to collect enough cells for two ABMTs. Patients with Multiple Myeloma that were collected prior to 2009 and then relapsed, did not have cells in storage.

Exposure to alkylating agents such as Melphalan is a risk factor for poor mobilisation. The introduction of Plerixifor which was first used at Nepean in 2011, enabled successful collection of Peripheral Blood Stem Cell Collection (PBSCC) for those without cells in storage. The first attempt at PBSCC for RMM was made in 2012. Between 2012 and 2015 PBSCC was attempted on a total of 6 patients.

Conclusion:

In the majority of cases it was found that time it took for mobilisation to occur was significantly delayed, peripheral CD34 results and subsequent dose collected were also significantly reduced compared to initial PBSCCs. However the majority of patients were able to achieve a dose sufficient for salvage ABMT, making it possible to effectively collect PBSCC post HD Melphalan and ABMT for RMM. Plerixifor was required in most of the cases justifying the use of pre-emptive dosing of Plerixifor. Mobilisation with HD Cyclophosphamide can make PBSCC mobilisation more successful, however there are risks from severe toxicities.

551. Compassion fatigue and burnout within cancer nursing

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Aim:

To ascertain Compassion Fatigue and Burnout rates within cancer nursing- A point prevalence study at a large metropolitan hospital, Sydney Australia.

Method:

A quantitative study was undertaken using The Maslach Burnout Inventory (MBI) assessment tool. Forty voluntary participants within the cancer nursing speciality were assessed using the validated tool. The survey instrument consisted of twenty-two questions which comprised The MBI survey. The researcher obtained a license to reproduce the questionnaire and the manual to interpret and scale results.

Result:

Varying levels of compassion fatigue and burnout were self reported in all three of the MBI's sub categories: Emotional exhaustion, depersonalisation and personal accomplishment. In this specific cohort participants with 6-10 years cancer experience reported highest levels of burnout and fatigue. The research collected during this project showed 82.5% (n=33) of participants state feeling lethargic and fatigued during their professional practice and 65% (n=26) describe a decrease in motivation to attend work. Within the group surveyed of cancer nurses, 62.5% (n=25) answered they perceive their workload as not sustainable and the primary factor contributing to their compassion fatigue and burnout.

Conclusion:

Compassion fatigue and burnout pose a serious risk to the sustainability of the nursing workforce. This research study has shown that while our cancer nurses are in fact displaying signs of compassion fatigue and burnout they are proactive in their quest to combat this alignment. Nurses with varying levels of expertise within cancer nursing are displaying signs of compassion fatigue and burnout. Further research is recommended to ascertain the impact of both fatigue and burnout on quality of care and patient outcomes.

552. Electrolyte Changes During Stem Cell Harvesting: Correcting the imbalance.

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¹SCGH, Nedlands, Australia

During stem cell harvesting (SCH) patients are at risk of procedure related side effects, mainly vasovagal reactions or symptoms related to hypocalcaemia and are monitored for symptoms throughout the procedure. A female patient with multiple myeloma (MM) suffered a critical incident following the processing 5 blood volumes (BV) for SCH. She became haemodynamically unstable and The Medical Emergency Team (MET) was called. Bloods results at the time of the incident revealed hypomagnesaemia and hypokalaemia and the patient required admission into the High Dependency Unit. This incident led to research into the effect that stem cell harvesting has on electrolytes.

Method:

Electrolytes and Serum Creatinine were measured at baseline and again following the processing of 2 BV for SCH. Sampling occurred on 25 patients (15 males, 10 females) over 30 SCH days; 18 patients with MM (21 SCH days), 5 patients with NHL (6 SCH days) and 2 other (3 SCH days).

Results:

After the processing of 2 BVs, 20 (80%) patients were hypokalaemic. Four (16%) patients received intravenous (IV) K⁺ and 8 (32%) others received oral supplementation. Hypomagnesaemia was recorded in 2 patients at baseline and 6 (24%) at reassessment (4 received oral supplemental 2 IV). 4 of those 6 were also hypokalaemic. Corrected calcium levels were preserved and no patients were hypocalcaemic on reassessment testing. Serum creatinine levels decreased overall during the course of SCH.

Conclusions:

Patients remained haemodynamically stable however electrolyte fluctuations were noted and corrected in our patient cohort.

Patients mobilised for SCH following multi-agent chemotherapy (e.g. relapsed NHL) or with refractory MM are at higher risk of electrolyte disturbances during SCH. It is therefore appropriate to reassess electrolytes during SCH. Our own practices have changed to reflect these conclusions by applying a practice guideline for electrolyte replacement and limiting SCH to 3-4BV/ day.

553. A specialist haematology unit audit of MDS patients in the ambulatory setting.

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Introduction:

The amalgamation of the Royal Melbourne Hospital and Peter MacCallum Cancer Centre haematology departments has created a specialist centre managing a large population of patients with myelodysplastic syndrome (MDS) and analysis of this cohort can better inform management policies and resource allocation.

Aim:

To review the prevalent MDS population and their treatments in our single clinical service.

Methods:

We undertook a cross-sectional audit of the current management of patients with MDS in the ambulatory setting. Data was accessed and aggregated from written and electronic records of MDS patients who were receiving treatment or ongoing follow-up between January 2011–December 2016.

Results:

289 patients were identified with a median age of 67 (range 17-90) years at diagnosis. 97 (33%) patients had died. 53 (18%) were treated with azacitidine either through a clinical trial or PBS (median duration 6 months, max 52 months); of these, 6 received azacitidine post-allogeneic bone marrow transplant (BMT) for relapsed disease (5 patients had pre-allograft exposure to azacitidine) 2 patients received it as a bridge to transplant. 69 (23%) median age 53 (range 17-72) underwent allogeneic BMT, of which 13(28%) relapsed at a median disease free survival of 242 days post-transplant. 71 patients (25%) were managed with a combination of supportive care/darbepoetin alfa and/or lenalidamide; of which 17 received iron chelation. 39 (13%) had red cell transfusions alone; median age 75 (56-90). 7 (2%) had "5q-syndrome" and were treated with transfusions and lenalidomide; all achieved transfusion independence.

Conclusion:

The complex and heterogeneous nature of this patient cohort and their different needs require an individualised approach. A patient-centred and multi-disciplinary approach should be adopted to meet their changing requirements specifically at time of disease progression. Access to clinical trials is particularly relevant for patients who have failed hypomethylating agents as their prognosis remains poor.

554. Central venous access device registries: improving outcomes for haematology patients

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Aim:

Central venous access device (CVAD) registries allow clinicians to examine device-related complications and patient outcomes over time. This registry collected information about the patients requiring CVADs within Cancer Care Services (CCS), type of CVADs being inserted, any complications and reasons for removal. By capturing this data, we have been able to identify variances and trends in the quality of CVAD care provided.

Primary outcome:

- To examine the feasibility of maintaining a CVAD Registry by pilot testing a peripherally inserted central catheter (PICC) database in CCS.

Secondary outcomes:

1. To measure the rate of CVAD failure per 1000-catheter and-patient days, due to infection, occlusion, thrombosis, fracture, migration and accidental dislodgement.
2. To calculate the average CVAD dwell time for patients receiving cancer care services.

Method:

The CCS PICC database captured information on all adult patients who received a PICC insertion from 1st April 2016 until 31st March 2017 at a quaternary referral hospital in Australia.

Result:

Five hundred and eighty nine CVADs were inserted in CCS patients. The majority of these CVADs were inserted for haematology patients (65%). Three hundred and twenty four PICCs were inserted into 257 patients. Two thirds of patients required one CVAD during treatment. One patient required eight CVADs. More than half (56%) had their CVADs removed due to CVAD-related complications. A quarter of patients had their CVAD removed due to suspected CVAD-associated bloodstream infection (26%). Such high CVAD failure rates are unacceptable and have resulted in significant costs to patients and healthcare providers.

Conclusion:

Timely solutions are needed to reduce CVAD failure and the associated healthcare costs. The CVAD Registry provides high quality local evidence on the risk factors and reasons for CVAD failure to inform the planning and evaluation of healthcare services.

555. Management of psychosocial issues associated with sickle cell diseases

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Sickle cell disease (SCD) is the most dominant inherited autosomal recessive disease in the world and belongs to a group of conditions known as haemoglobinopathies. Approximately 300,000 children are born with sickle cell disease every year worldwide, and 80% of these births occur in poor socio-economic countries such as sub-Saharan African and the Middle East. Reports have shown that carriers can also be found in some European countries and small ethnic groups in India¹. Due to migration, a sizeable sickle cell population is now emerging in traditionally unaffected areas of the world.

The medical management of SCD is standard: pain management, blood transfusion, red cell exchange, Hydroxurea and in some cases stem cell therapy. Research has shown, however, that these patients also deal with poor Quality of Life (QOL) facing stigma, discrimination, low self-esteem, lack of specialty care, social isolation and significant risk of early mortality¹.

As SCD is common in only a small percentage of individuals compared to other haematological disorders, health care workers (HCW) sometimes struggle to deal with these psychosocial issues. Management of SCD patients can be challenging to HCWs with some patients perceived as being difficult to deal with and having attention seeking behaviours. The HCW's improved understanding of the experiences of SCD patients will help facilitate more effective delivery of quality care.

Little research has been undertaken related to psychosocial issues, spirituality and QOL of the SCD patients.

This poster will present the results of a literature review focusing on the psychosocial issues of SCD patients and ways for the HCW to develop better understanding of SCD, utilising emotional intelligence and empathy in order to improve quality holistic care for SCD patients. Gaps in the available literature are highlighted and recommendations made for further research in this area.

No conflict of interest to declare

- Mann-Jiles, V & DL Morris (2009) Quality of life of adult patients with sickle cell disease. *Jnl American Acad of Nurse Practitioners*, 21, pp.340 – 349.

557. Exercise promotion during chemotherapy treatment: recommendations for the Australian oncology nurse

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Exercise has proven physiological and psychological benefits when undertaken regularly and with appropriate guidance. The understanding of exercise and its effect on adults with a diagnosis of cancer can be misunderstood and is still evolving. Oncology nurses are in frequent contact with people receiving chemotherapy for cancer, placing them in an ideal position to educate and empower patients to begin or maintain regular exercise. This paper identifies the benefits of exercise in adults with a diagnosis of cancer receiving chemotherapy, types of exercises to recommend to patients, potential barriers to patient compliance, information about exercise practitioners, and considerations and contraindications. The potential for improved health outcomes through exercise intervention is undeniable. Oncology nurses are vital for increasing awareness and providing practical advice to patients undergoing chemotherapy treatment.

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558. The Australian CAR Industry- local manufacture of CAR T-cells for B-cell malignancies

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With promising results from clinical trials in B-cell acute lymphoblastic leukaemia, non-Hodgkins lymphoma and multiple myeloma, CAR T-cells are set to revolutionise the treatment of haematological malignancies. Australian cell therapies laboratories will potentially play a role in the collection and transport of raw materials and receipt and distribution of the final CAR T-cell product. However, laboratories also have the opportunity to be more than passive conduits for the pharmaceutical industry, but to utilise their expertise and engage fully in the development and production of CAR T-cells for localised use. Laboratories wishing to do so will need to embrace the relevant regulations and engage with local health networks and government bodies to ensure the appropriate staff and resources are in place. Given the likely expense of highly modified immune effector cell products investment in this space will enable the provision of these therapies at a significant saving to the public health system in Australia now and into the future.

559. Adoptive T cell therapy approaches to tackle GVHD

Tey S

In vitro or in vivo T cell depletion is effective in reducing the incidence of graft-versus-host disease (GVHD) but comes at a cost of impaired immune reconstitution with consequently increased risks of infection and leukaemia relapse. The dose of T cells that can be safely infused varies between donor-recipient pairs and is not always predictable. The therapeutic window is particularly narrow in highly HLA-mismatched transplants. T cells can be gene-modified with a 'safety switch', which enables their conditional elimination in the setting of adverse events, such as life-threatening GVHD. We will present our institutional experience using T cells carrying the inducible caspase 9 (iCasp9) safety switch in adult patients undergoing haploidentical transplantation. We will also present our ongoing work in developing regulatory T cell (Treg) therapy for patients with established chronic GVHD, and the potential utility of gene-modification as a means to track the in vivo fate of adoptively transferred Tregs.

560. Mesenchymal Stromal Cells: Clinical manufacture and trials

Sturm M

Mesenchymal stromal cells (MSC) have broad therapeutic potential through their ability to modulate immune and inflammatory responses and also to induce immune tolerance. Their multipotency has seen their application to tissue regeneration. Their hypo-immunogenicity and inability to elicit an immune response means they are a universal donor cell and potentially available “off the shelf”. However, the translation of MSC as a mainstream therapy has been slow, due in part to challenges of manufacturing and also as a result of disappointing Phase III data from the commercial sector.

Cell & Tissue Therapies WA (CTTWA) at Royal Perth Hospital has been manufacturing allogeneic, bone marrow derived MSC since 2007. Manufacture complies with the Australian code GMP and the relevant therapeutic goods orders. Despite regulatory challenges, CTTWA obtained a TGA manufacturing licence for MSC in 2013, to progress Phase II clinical trial evaluation. FACT-JACIE accreditation for MSC manufacture was obtained in 2016.

CTTWA has manufactured for 11 clinical trials or studies, 7 of which are complete and 4 of which are recruiting. All but one are involved in immune/inflammatory disorders. This includes adult and paediatric graft versus host disease, organ transplant rejection (lung and kidney), chronic obstructive pulmonary disease and myelodysplastic disease. The other trial is in bone regeneration for cranioplasty. MSC have also been provided on compassionate grounds. For immune/inflammatory disorders, patients receive infusions of 2×10^6 MSC/kg patient weight, for 2 or 4 infusions at various intervals. More than 136 patients have received MSC therapy, in over 586 infusion episodes. No infusion related adverse events have been observed. Many patients are more than 5 years post initial treatment, with the longest approaching 10 years. Some patients have received multiple treatments for relapses. Initial studies for the various indications were early phase, non-randomised studies in severe clinical cohorts, refractory to other treatments. The outcomes of completed early studies has been very encouraging and has driven Phase II randomised, multi-sited trials, in some indications.

For full development of MSC therapy, Phase III, randomised, blinded, comparative, multi-sited clinical trials in key indications are indicated but require significant investment.

561. Myeloid leukaemia specific T cell production

Blyth E
Westmead Hospital

Abstract not supplied

562. T cell banks for the treatment of viral infections

Clancy L

Abstract not supplied

563. TCR α + β +/CD19+ cell processing & flow analysis

Hutchins C

Abstract not supplied

564. TCR $\alpha\beta$ + / CD19+ cell depleted haploidentical stem cell transplantation for paediatric patients

Mitchell R

Abstract not supplied

565. Results of using automated CliniMACS Prodigy for CD34 selection from mobilized peripheral blood stem cell products

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Cell selection is an important part of graft manipulation for cellular therapies. The CliniMACS Plus system has been used in our laboratory for over 15 years to deplete T-cells from stem cell grafts of the unrelated donors for paediatric patients undergoing BMT. The recently introduced CliniMACS Prodigy® (Miltenyi Biotec) permits automated selection of CD34+ cells from mobilized peripheral blood stem cells (PBSCs) using monoclonal antibodies conjugated to paramagnetic particles in a complete closed system. We have been evaluating the Prodigy and we report the most extensive evaluation, to date, between the Prodigy and the CliniMACS Plus systems.

Methods:

We validated the CD34 selection program on the Prodigy (LP-34, using the new version 2.0) against the standard semi-automatic CD34 selection process on the CliniMACS Plus. PBSCs were collected by apheresis from nine healthy adult subjects after mobilisation with G-CSF. Excess PBSCs were loaded onto the Prodigy the day after the clinical procedure. The nine Prodigy procedures were assessed for product quality against historical CliniMACS Plus data. Samples used for the Prodigy validation (not clinically infused), were only used when the requested CD34 dose had been achieved using the CliniMACS Plus.

Results: (see Table 1):

Post selection cell parameters Median value (range)	CliniMACS Prodigy N=9	CliniMACS Plus N=66	P Value (2-tailed unpaired t-test, equal variance)
Procedure time (mins)	300	330	N/A
"Hands on" operator time (mins)	90	240	N/A
% viable CD34 Purity	76.0 (57-90)	82.0 (52-96)	P=0.1152
% viable CD34 Recovery	67.0 (64-87)	69.2 (46-91)	P=0.4953
CD3 Depletion (log)	4.4 (4.3-4.8)	4.8 (2.9-5.8)	P=0.0117
CD3 x10 ⁴ /kg	0.6 (0.1-2.9)	1.0 (0.08-14.4)	P=0.2689
CD19 Depletion (log)	3.3 (3.2-3.5)	3.7 (2.3-4.7)	P=0.0099
CD19 x10 ⁴ /kg	1.5 (0.6-5.0)	3.2 (0.3-38.4)	P=0.1966

Conclusion:

CD34+ cells can be effectively selected from mobilized PBSCs with the CliniMACs Prodigy, including those that have had prolonged transit times. The recovery and purity of CD34+ cells was comparable to the CliniMACS Plus but depletion of T and B-cells was found to be lower on the Prodigy compared to the CliniMACS Plus. The T cell and B cell content per kg, from the Prodigy, was below 10x10⁴/kg, the upper limit for clinical release criteria. The Prodigy offers a single device for CD34 cell selection, unlike the CliniMACS Plus which required the apheresis product to have prior processing (such as platelet removal, labelling and washing prior to cells etc.) on other equipment. The Prodigy process is fully automated and this translates into a reduction in the time required of experienced laboratory staff. Our results suggest that the Prodigy can be used for the routine clinical application of CD34 selection to HSCT products.

566. Standards and Regulations for HPC/Cellular Therapy

Trickett A

The accreditation and regulatory requirements applicable to cellular therapy programs are complex. For many years, Australian facilities that collect and/or process haemopoietic progenitor cells (HPC) for transplantation have been required to attain NATA accreditation. Paediatric centres that participate in the Children's Oncology Group (COG) clinical trials must obtain international accreditation through the Foundation for Accreditation of Cellular Therapy (FACT), whereas Cord Blood Banks and manufacturers of cellular therapies that are included in the TGA Biologicals Framework are required to obtain a TGA licence.

Each accreditation or regulatory body requires adherence to a different set of standards, requirements and/or guidelines. This presentation will aim to give an overview of the current regulatory requirements plus recent changes and updates in this field. The talk will include information on the TGA regulation of Biologicals and the new FACT standards for Immune Effector Cells, which includes dendritic cells, natural killer cells, T cells, B cells, genetically engineered chimeric antigen receptor T cells (CAR-T cells) and therapeutic vaccines.

567. A comparison of body weight for calculating CD34+ cell dose in an autologous transplant facility

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A review of the current practice of using Ideal Body Weight (IBW) and Actual Body Weight (ABW) in calculating CD34+ cell dosage for patients undergoing autologous haematopoietic progenitor cell (HPC,A) collection was conducted following a routine NATA accreditation after noticing increasing trends in obesity, in order to maximize resource utilization. A retrospective study was performed in order to validate the use of Adjusted Body Weight (AdjIBW) to calculate the CD34+ cell dosage for patients considered to be obese (patients with a $\geq 25\%$ difference between IBW and ABW). All autologous stem cell transplants performed at the Princess Alexandra Hospital (PAH) between January 2011 and May 2017 were reviewed to ensure the AdjIBW equation is applicable to transplant patients and there is no significant effect on a particular group of patients e.g. low weight female myeloma patients or obese patients. Our target cell dose is $5 \times 10^6/\text{kg}$ (IBW), and a minimum of $2 \times 10^6/\text{kg}$ (IBW) as the primary endpoint of collection. 217 autologous stem cell reinfusions were reviewed (144 males, 73 females). 104 patients (47.9%) weighed $\geq 25\%$ more than their IBW, and as literature suggests should therefore have AdjIBW calculated. The median actual difference between their IBW and ABW was +41% (+25% - +158%), with a median ABW of 92kg (57kg – 176kg) and IBW of 64kg (42kg – 88kg). Using the formula $\text{AdjIBW} = \text{IBW} + 0.25(\text{ABW} - \text{IBW})$ as per Hicks et al (2012) adjusted body weights were calculated. The AdjIBW for this cohort of patients is 72kg (46kg – 103kg). All patients engrafted with no difference between median times to neutrophil and platelet engraftment when compared to the patients $\leq 24\%$ difference between IBW and ABW (11 and 18 days respectively). Further analysis is underway to determine the effect of using IBW or AdjIBW for obese patients undergoing autologous stem cell mobilisation. Meanwhile we take into consideration all body weights (IBW, ABW and AdjIBW) when determining an end point for HPC,A collection to optimize patient outcome while maintaining an effective use of resources.

No conflict of interest to disclose.

569. Allogeneic Stem Cell Transplantation (ASCT) as a Risk Factor for Recurrent Melanoma

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Aim:

There is a well-documented increased risk of secondary malignancies following ASCT but the risk of recurrence of prior malignancies diagnosed prior to transplant remains ill-defined, including melanoma which is known to be an immunoresponsive tumour. We describe two cases of recurrent melanoma after ASCT which suggest that the natural history of melanoma may be adversely affected in this setting.

Results:

A 48-year-old woman was diagnosed with CLL and during the following 8 years received therapies including fludarabine, cyclophosphamide and rituximab and a phase 1 trial with a bcl-2 antagonist (navitoclax). Eight years following CLL diagnosis, the patient was diagnosed with stage 1B melanoma treated with excision. Fourteen months post melanoma diagnosis, progressive CLL necessitated the need for reduced-intensity ASCT. Three months following transplant, new skin lesions developed and excision confirmed these as melanoma, which metastasised and she died within weeks.

A 48-year-old male was diagnosed with CLL in 1999 and underwent multiple lines of chemotherapy over the following 10 years including cyclophosphamide, vincristine and fludarabine-containing regimens. Progressive disease occurred in 2010 leading to a haplo-identical sibling ASCT with subsequent mild skin graft-versus-host disease (GVHD). Six months prior to transplant a stage 1B melanoma was removed from his right mandible and 30 months post transplant progressive lymphadenopathy on PET scan was proven to be metastatic melanoma. Initial treatment of dacarbazine chemotherapy and radical neck dissection led to a negative PET scan. Peritoneal recurrence in 2015 responded to ipilimumab without reactivation of GVHD

Discussion:

Profound immunosuppression following ASCT may be a substantial risk factor for melanoma recurrence. Patients with a previous melanoma unequivocally requiring an ASCT should ideally receive the minimal immunosuppression that is clinically appropriate and rigorous post-allograft skin surveillance. Immunotherapy may potentially be safely given post-ASCT in the absence of significant GVHD if recurrence occurs

570. Prolonged transit time does not impair HPC CD34 selection and cryopreservation

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Aim:

To assess the effect of transit time on recovery and purity of CD34 selection.

Methods:

Healthy donor G-CSF-mobilized apheresis products and marrows were obtained from national and international donor centres. CD34 selection procedures were performed using the CliniMACS Plus (Miltenyi). The time between the end of apheresis and start of loading product onto the CliniMACS Plus was adjusted for time zone differences. Viable CD34 flow enumeration was performed before and after CD34 positive selection using ISHAGE gating.

Results:

Over a 10 year period, 54 products were received. 18 were from Sydney centres, 7 from national centres and 29 from centres overseas, mainly in Europe and North America. The majority of the products were HPC(A) (50/54). The time interval from end of collection to loading the cells onto the CliniMACS was 1 to 58 hours (median 32.1 hrs.). After CD34 selection, the median CD34 purity was 81% (range 54-96%). The median CD34 recovery was 67% (range 46-91%). There was no significant correlation between transit time with either CD34 recovery ($r=-0.006$, $p=0.967$, figure below) or purity ($r=0.224$, $p=0.104$). The median CD34 + cell dose/kg for infusion was 11.5×10^6 CD34/kg (range 2-33). In 9 cases a scheduled delay between receipt of cells and infusion meant cells were cryopreserved after CD34 selection. In these 9 the median thawed viable CD34+ recovery was 70% (range 55-90%).

Conclusion:

Our data shows that HPCs can be collected and transported across the world prior to CD34 selection followed by immediate allogeneic transplantation or cryopreservation. There was no significant difference in CD34% recovery and purity post-selection for transit times in excess of 24 hours, and this did not compromise the cells after a CD34 selection procedure. Such data is relevant as newer technology, such as gene & CAR-T cell therapies become more widely used for patients with overseas donors.