Blood 2023

ABSTRACT BOOK

2023 Annual Scientific Meeting

5 - 8 November

Melbourne Convention and Exhibition Centre

The combined Annual Scientific Meeting of the:

HSANZ
Haematology Society of Australia and New Zealand

ANZSBT
Australian & New Zealand Society of Blood Transfusion Ltd

THANZ
Thrombosis & Haemostasis society of Australia and New Zealand

Table of Contents

Carl de Gruchy Oration: Haematology: What a difference we can make!

Roberts A

In the 2023 Carl de Gruchy Oration, I would like to share with you my personal reflections on why Haematology is such a compelling calling. Haematology research has changed our understanding of the basis of many blood disorders, malignant and non-malignant, common and uncommon. Research has also delivered major improvements in our diagnostic and therapeutic armamentaria, and use of these in practice in 2023 is delivering much improved care and outcomes for many patients. But the most compelling thing about Haematology is that we are not resting on our laurels. We've made some big advances, but that should just whet our appetite to deliver more. In sharing some anecdotes of my time as a haematologist and haematology researcher, I'd like to highlight how the work we do in Australia and New Zealand can make differences felt around the world.

Ruth Sanger Oration: Turning the page on blood typing: Reflections on a classic text

Flower R

First published in 1950, the classic text “Blood Groups in Man” written by Ruth Sanger and her husband Robert Race, progressed through six editions over 25 years. Documenting the rapid development of a complex field was a mammoth undertaking that provided the basis on which the next generation of immuno-haematologists have built. A theme through the editions is immunohaematology as an international enterprise based on trust, communication and sharing.

The 290 page first edition covered eight blood group systems, by the sixth edition it had more than doubled in size and covered 15 blood group systems, in addition to many named antibodies now recognised as defining blood group systems. In the preface to the second edition, covering nine systems, the authors comment “there are so many blood group systems”. They may have been astonished that there are now 44!

Chapters in the early editions outlined the theoretical basis for procedures, now in universal use, to resolve the specificity of antibodies. Some of the investigations that were difficult in 1954 would be routinely resolved with the well-designed panels available today.

In the first edition, almost over a third of the text was devoted to the complexity of the Rh system, its discovery and inheritance, investigation methods and the range of antigens. The authors may have been amazed that high-throughput sequencing has revealed even greater complexity in this system.

I will discuss examples where further investigation has confirmed that a variety of named antibodies define the same antigen. The basis of these achievements remains international collaboration with sharing of data and samples. Although the baton of progress in immunohaematology is now passing to specialists in high throughput sequencing, the techniques defined in these texts continue to provide the bedrock of safe transfusion practice.

Addressing the digital divide: Mitigating health inequities

Watson L

Currently, inequities exist in patients' ability to access care, some of which are exacerbated by the health care system's embrace of new and innovative digital health solutions. As a result of the COVID-19 pandemic, health care organizations and community agencies quickly transitioned to delivering care, support, and education virtually, to ensure continued access to care and support for patients during the global public health crisis. Although public health restrictions have now been lifted, reliance on and utilization of these digital health solutions has remained, because patients, families, and communities continue to show interest in accessing care virtually, and because these solutions provide an effective way to manage health system barriers that prevent equitable access to care such as health human resource shortages and limited access to specialist care in rural communities. However, as technology advances and digital health solutions become more embedded into routine practice within cancer care, a purposeful approach to mitigate barriers to digital health care that are more prevalent in marginalized populations is necessary, or we risk further disadvantaging equity-deserving populations who have limited or no access to technology. Everyone deserves an equal opportunity to benefit from health technology. The digital divide in healthcare is real, but do we see it? How can we enact strategies to bridge the gap if we don't ask basic questions about access to technology, the internet, or their digital literacy? The fact that health systems does not ask these basic questions highlights our failure to provide the support people need to maximize their ability to engage in digital healthcare.

Is Autograft Dead in DLBCL?

Anderson M
Associate Professor Mary Ann Anderson MBBS, FRACP, FRCPA, PhD
Consultant Haematologist Department of Clinical Haematology Royal Melbourne Hospital and Peter MacCallum Cancer Centre

Autologous stem cell transplant (ASCT) has been the mainstay of therapy for fit patients with relapsed and / or refractory (rr) diffuse large B cell lymphoma (DLBCL) for the last three decades[1]. However, the well-recognised toxicities of this treatment meant that age and co-morbidities excluded many patients from proceeding to ASCT. Even among those who are fit for transplant failure to respond adequately to salvage meant that approximately 50% did not make it to ASCT[2]. Patients whose DLBCL relapsed post ASCT traditionally had a very poor prognosis.
The advent of chimeric antigen receptor T (CART) cell therapy has changed the outlook for patients with rr DLBCL. The Juliet study was the first global study of the autologous anti CD19 CART tisagenlecleucel in patients beyond second line therapy in rr DLBCL[3]. This study demonstrated a complete response (CR) rate of 32% at three months[3]. Similarly, the Zuma1 study of the autologous anti CD19 CART axicabtagene in patients with large cell lymphoma relapsed post ASCT demonstrated a CR rate of 58% [4]. These two pivotal studies underpin the current Australian indication for accessing funded CART in DLBCL that has relapsed post autograft or two prior lines of therapy.

Building on the promising results of CART therapy in difficult to treat rr DLBCL the Zuma 7 study randomised patients with DLBCL, that was refractory to or had relapsed within 12 months of front-line therapy, to either axicabtagene or 2-3 cycles of platinum based chemoimmunotherapy followed by ASCT[5]. This study demonstrated and overall survival advantage in favour of the axicabtagene arm, even despite the majority of patients with rr disease on the standard of care arm receiving subsequent off protocol cellular immunotherapy (57%)[5]. The ongoing Zuma-23 study is examining the role of CART in the front line for high-risk large cell lymphoma patients (NCT05371093).
Autologous anti CD19 directed CART has now demonstrated a survival advantage over salvage and autograft when used in the second-line setting. This survival advantage combined with the favourable toxicity profile of CART cell therapy over ASCT make CART cells an attractive option for DLBCL patients in the second line of therapy. The key challenge in realising the potential of this therapy in the Australian and New Zealand setting is likely to continue to be the very high-cost nature of the treatment.

Disclosures

MAA is an employee of the Walter and Eliza Hall Institute that receives milestone payments in relation to venetoclax to which she is entitled to a share. MAA declares honoraria from AbbVie, Jansen, Beigene, AstraZeneca, Gilead, Novartis, Roche, Takeda, CSL

References

1. Friedberg, J.W., *Relapsed/refractory diffuse large B-cell lymphoma.* Hematology Am Soc Hematol Educ Program, 2011. **2011**: p. 498-505.
2. Philip, T., et al., *Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma.* N Engl J Med, 1995. **333**(23): p. 1540-5.
3. Schuster, S.J. and J. Investigators, *Tisagenlecleucel in Diffuse Large B-Cell Lymphoma. Reply.* N Engl J Med, 2019. **380**(16): p. 1586.
4. Locke, F.L., et al., *Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial.* Lancet Oncol, 2019. **20**(1): p. 31-42.
5. Westin, J.R., et al., *Survival with Axicabtagene Ciloleucel in Large B-Cell Lymphoma.* N Engl J Med, 2023. **389**(2): p. 148-157.

Using PROMs to assess transfusion outcomes in patients with myelodysplastic syndromes (MDS)

Mo A

Patients with myelodysplastic syndrome (MDS) frequently have anaemia, which is associated with impaired quality of life (QoL). Transfusion is given to alleviate symptoms of anaemia and improve QoL, however the evidence guiding optimal transfusion practice in these patients is sparse.

In this session, trial data and current literature relating to the use of PROMs in MDS patients will be explored, including whether PROMs can be used to assess transfusion outcomes in this patient cohort. The potential use of PROMs in the real-world setting to guide transfusion practice will also be discussed.

Integrating electronic Patient Reported Outcomes (ePROs): Exploring the benefits to patients, clinical teams and the system

Watson L

Cancer patients experience numerous distressing symptoms and concerns across the course of their illness, which negatively influence their quality of life. Regardless of cancer type, unmanaged symptoms can lead to adverse downstream consequences such as the need for unplanned urgent care either in the Emergency department or acute care hospital. These unplanned visits can be costly to the healthcare system and distressing for patients. Patient Reported Outcome Measures (PROMs) in the ambulatory care space can be used to inform targeted symptom management and proactive patient care in the lower cost environment that can decrease more costly health system encounters. However, simply gathering this information from patients does not improve outcomes. Patient generated information about symptoms and concerns must be routinely collected, be presented to the clinical team in an easy to access and interpret format, and utilized by the clinical team to target symptom management and interventions if it is to impact health system outcomes in ambulatory oncology facilities and the broader health system. In this presentation, a case example will be shared highlighting how ePROs have been fully integrated into routine care at one Canadian provincial ambulatory cancer program. Using ePROs routinely in this provincial practice settings has had numerous benefits including enhanced patient-clinician communication, assisting with problem detection, management of symptoms, and improving outcomes for patients. Provincial data also shows strong correlation between self-reported high symptom complexity and the unplanned use of acute care services within 7 days of ePRO completion, demonstrating the utility of ePROs as a strategy to identify patients who would benefit from additional targeted supports in the lower cost ambulatory setting.

Management of haematological malignancies in pregnancy

Kidson-Gerber G

The diagnosis of lymphoma or leukaemia in pregnancy is a complex and challenging situation. With the goal of delivering optimal curative therapy, the unique risks to the mother and developing fetus must be considered. Dr Kidson-Gerber will present some cases and the current data which informs the approach to the diagnosis, imaging and management of women presenting with lymphoma and leukaemia in pregnancy.

Transfusion 2024 (+10) – How will we be thinking about transfusion in the 2030s?

Charlton A

In England we are making our way through an ambitious national programme of change and improvement in transfusion practice (Transfusion 2024) that was originally planned to complete next year. But how will we be practising transfusion medicine 10 years from then and beyond? We will consider the role of donor and patient genotyping, a new level of 'universality' of components, new thinking about logistics and transport, and other topics.

How will we be managing inherited cancer predisposition syndromes in 10 years time?

Godley L

During this session, Dr. Godley will predict what our cancer predisposition testing will look like in 10 years' time. Cancer risk screening will likely be largely centered on tumor-based testing as molecular testing of tumors is used increasingly for diagnostic, prognostic, and therapeutic purposes. Our bioinformatic pipelines will become more accurate in predicting which DNA variants, both single nucleotide and copy number variants, found in tumor-based profiling are actually germline alleles. Testing for germline predisposition to cancer will likely become routine for patients with hematopoietic malignancies, as are flow cytometry/cytogenetic/molecular testing currently. Testing of potential allogeneic hematopoietic stem cell donors will also likely become routine regardless of whether a donor is related to the patient or not, given the presence of many cancer predisposition alleles within populations: founder mutations in particular populations, like is seen for *BRCA1/2*; as well as other cancer predisposing alleles found in general population databases, as is seen for *CHEK2* and *DDX41*. Treatment of patients may be altered in the future based on germline cancer risk alleles. For example, we recognize now that individuals with deleterious germline *DDX41* variants are at risk for developing severe graft versus host disease after allogeneic hematopoietic stem cell transplant, even with wild-type donors, a complication that can be alleviated with the use of post-transplant cyclophosphamide. On-going work is likely to shed light on the frequency of deleterious germline variants in the unrelated donor pool, a result that could inform future recommendations to test all allogeneic hematopoietic stem cell donors regardless of their relationship to the patient. Future studies are also likely to inform us on whether these alleles in the patient/hematopoietic stem cell recipient and/or donor affect other poor outcomes from allogeneic transplant, like graft failure and donor-derived hematopoietic malignancies; as well as how clonal hematopoiesis in the donor impacts allogeneic transplant outcomes. The use of allogeneic hematopoietic stem cells with deleterious germline variants and/or clonal hematopoiesis may depend ultimately on the age of the transplant recipient, with younger patients who have longer life expectancies potentially in greater need of cells that lack these variants/conditions.

Time-limited vs ongoing therapy for CLL

Brown J

Development of CLL targeted therapy began with the approval of the B cell receptor pathway inhibitors, in particular the BTK inhibitors, which were developed largely as single agents to be given as continuous therapy. As these regimens moved to earlier lines of therapy, it became increasingly clear that continuous therapy over years had undesirable consequences, including the accumulation over time of toxicity and cost, as well as the risk of selecting resistant clones. Therefore, clinical development efforts shifted to focus more on time-limited targeted therapy, beginning with the one year venetoclax obinutuzumab (VO) regimen developed in the CLL14 study, and since then moving on to combined BTK inhibitor (BTKi) – venetoclax regimens that have been studied as fixed duration or minimal residual disease (MRD) guided regimens. With longer follow-up of all of these studies, some themes have begun to emerge. The first is that, after time-limited therapy, disease with higher risk biology tends to relapse earlier, just as we saw in the era of FCR chemoimmunotherapy. Hence in CLL14, patients with 17p deletion relapse with the shortest median PFS, while those with unmutated IGHV also have a reduced median PFS compared to those with mutated IGHV, who may have very prolonged remissions that are ongoing. Also similar to what we saw with time-limited chemoimmunotherapy, achievement of undetectable MRD is very predictive of outcome after time-limited VO, whereas it is much less predictive if patients remain on continuous therapy with BTK inhibitors, which appear to be effective in maintaining quiescence of residual disease. Even TP53 aberrant disease can have prolonged remissions on continuous BTK inhibitors – although these remissions still appear shortened compared to patients without TP53 aberrancy. Data are only starting to emerge on the BTK inhibitor-venetoclax combinations, but so far it appears that, when truly fixed duration, the biologic prognostic factors again predict durability of response, but achieving undetectable MRD may not be quite as predictive of progression-free survival as with VO. Interestingly, rates of undetectable MRD in patients with mutated IGHV are lower after BTKi-venetoclax combinations than they are after venetoclax obinutuzumab, although implications for progression-free survival are not yet clear. Many outstanding questions remain, and include patient selection for the different types of time-limited therapies, optimizing the duration of time-limited therapies, and understanding the potential benefit of re-treatment with the same time-limited therapy at relapse.

Flow cytometric monitoring of lymphoid malignancies following antigen-directed immunotherapies

Chan K

Multiparametric flow cytometry is an essential component of the diagnostic haematology armamentarium, enabling rapid quantification and detailed immunophenotyping of haematopoietic cells across a broad range of clinical contexts. Following progressive technical and methodological advances, flow cytometry is now routinely used for quantification of measurable residual disease (MRD) in acute leukaemias, plasma cell myeloma and lymphomas, with established prognostic benefit for patients who achieve MRD-negative remissions confirmed at high assay sensitivity (typically a minimum of 10^{-4}).

MRD analysis relies on identification of neoplastic cells through expression of ubiquitously expressed, lineage-defining antigens, as well as aberrant expression of other markers that enable them to be distinguished from their normal counterparts. Consequently, analytical challenges arise when shifts in disease immunophenotype occur, potentially affecting the ability to reproducibly isolate and quantify MRD. This is a particular issue given the rapidly expanding utilisation of potent immunotherapies such as bispecific antibodies and chimeric antigen receptor T (CAR-T) cells, which often target key lineage antigens and can promote antigen-negative relapse. This presentation will provide an overview of MRD analysis in lymphoid malignancies and highlight potential pitfalls in the current era of antigen-directed immunotherapies.

Immunotherapy for B-cell lymphoma

Cheah C

Many of the most potent agents recently approved and in development for the management of patients with B-cell lymphoma are immunotherapies. These include cellular immunotherapies such as chimeric antigen receptor T-cells, bispecific T-cell engaging antibodies, immune checkpoint inhibitors and antibody drug conjugates. A summary of the key data for these agents in patients with B-cell lymphoma will be presented, along with suggestions on how these agents may be integrated into clinical practice.

Germline predisposition to malignancy

Godley L

In this session, Dr. Godley will describe current algorithms to identify patients/families likely to have germline cancer predisposition alleles, with a focus on risk of developing hematopoietic malignancies. She will review options for testing platforms and the genes that should be considered depending on the personal/family history of the presenting patient. Dr. Godley will also discuss the identification of potential cancer risk alleles from tumor-based molecular profiling. Dr. Godley will review the inclusion of inherited predisposition disorders within the diagnostic criteria of numerous classification schema as outlined by the World Health Organization, European LeukemiaNet, National Comprehensive Cancer Network, and the International Consensus Classification.

Dr. Godley will highlight her recent work on delineating the frequency with which deleterious germline variants cause myelodysplastic syndrome (MDS) and aplastic anemia (AA). Her group has shown that in those diagnosed at age 40 or younger, 19% of individuals with MDS/acute myeloid leukemia (AML) with prior history of MDS and 15% of those with AA have such alleles. In a more recent study, her group collaborated with the Center for International Blood and Marrow Transplant Research to estimate that the frequency of deleterious germline predisposition alleles is at least 7%, with positive cases spread across the age spectrum. Prior work using a large cohort of children with MDS, LP/P germline variants in *SAMD9/SAMD9L* were identified 8% and in *GATA2* in a mutually exclusive 7%. The age of presentation of MDS differed in these two genetic cohorts with *SAMD9/SAMD9L* prevalent in younger children and *GATA2* more common in older children. Thus, the age at which MDS/AA is diagnosed is linked to the underlying biological pathway(s) driving hematopoietic cancers, with variants in genes encoding transcriptional regulators common in children, DNA repair and telomere biology genes dominating adulthood diseases, and *DDX41* frequent in the elderly.

Haemovigilance - Lessons learned from STIR

Akers C

Blood Matters Serious Transfusion Incident Reporting: Lessons learned

STIR is a voluntary haemovigilance program run by Blood Matters in Victoria and accepts reports from four jurisdictions through memorandums of understanding. Annual reports of clinical and procedural events are published, including case studies and key messages for health services to review their processes and potentially make improvements.

The lessons learned from STIR include, but are not limited to the following:

- All incidents need investigation. Where policies are not followed it is important to understand why, so improvements can be made to prevent reoccurrence.
- Appropriate transfusion. An unnecessary transfusion that leads to a rection or incident is an avoidable event.
- Careful pre-transfusion assessment, especially for high-risk patients can reduce occurrence or recurrence of some reaction types.
- Electronic systems, including electronic medical records, need to be monitored to ensure we do not introduce new areas for error.
- Wrong blood in tube events continue to occur regularly and highlight some of the patient/procedure identification issues faced in health services.
- Transcription errors have been seen particularly in maternity services where there often seems to be transcription of results to the woman's medical record or in letters to the health service. This has led to missed RhD immunoglobulin doses for these women.

Errors and reactions continue to occur. Many errors may be able to be eliminated by better understanding and closing the gap between the work as imagined in policies and procedures compared to the work as done in the clinical environment.

Changing to one national health service

Archer G

New Zealand is one year into the plan to unite 20 District Health Board into one Te Whatu Ora or “weaving of wellness”. Being the first Patient Blood Management (PBM) service in New Zealand we have had the luxury of breaking down barriers in the administration of blood and blood products and of working with New Zealand Blood Service (NZBS). Now we are having to negotiate that pathway with 20 different Health Boards and find ways to collaborate and standardise what we do and how we work with NZBS to maintain standards of patient care.

Implementation of a new blood draw method

Archer G

When a Jehovah's Witness patient was admitted to our oncology/haematology ward with a new diagnosis of Acute Promyelocytic Leukaemia (APML) it caused some consternation amongst the clinical staff. Questions were asked about how we would get him through a course of treatment of his potentially curable leukaemia without the use of blood products. Once a way to minimise the amount of blood taken from him with each blood draw was discovered the challenge was how to ensure the patient was happy with the plan, the nursing staff were able to implement it and also that the quality of the blood draws taken would be upheld. This is how we did it.

Transfusion in haem-onc – current challenges

Charlton A

Despite the considerable demand for blood products to support the improvements in outcomes for many haem-oncology patients, there is relatively little high-grade evidence on which to base transfusion practice in the area. We will consider what evidence there is, efforts currently underway to bolster this, and areas of challenge and controversy in managing these patients.

Check twice, label once!

ChoiMaxwell S

A campaign launched in New Zealand in November 2022 to standardise the labelling criteria of pre transfusion samples in NZBS Blood Banks.

Under the current policy, roughly two percent of samples sent to NZBS Blood Banks are mislabelled samples that can be corrected while another two percent are rejected immediately. We receive a wrong blood in tube (WBIT) several times a month. This has the potential for the wrong blood product being issued to the patient, causing an ABO-incompatible transfusion. And we know that mislabelled samples have a more than 100-fold increased risk of being a WBIT.

NZBS made a policy decision to move to more restrictive criteria for pre-transfusion testing samples in order to improve safety and quality standards for sample acceptance criteria. The new policy is intended to ensure samples are unequivocally traceable to an identified patient and therefore reduce the number of wrong blood in tube samples received across New Zealand.

Nearly a year on. We have looked at how well the new policy is embedded in hospitals and if labelling practice has improved.

Australian Fresh Frozen Plasma Audit: A National Blood Transfusion Committee and Blood Matters Collaboration

Clarke L

Aim: To gain an understanding of fresh frozen plasma use across Australian health services and determine if use is in accordance with current guidelines.

To identify educational opportunities on the appropriate use of FFP to facilitate practice change.

Method: The FFP audit was developed by the National Blood Transfusion Committee (NBTC) in conjunction with Blood Matters Victoria and utilised the Australian Red Cross Lifeblood Audit Tool. Retrospective data was entered by Australian health care providers capturing FFP transfusion within the 12 month period of 1st April 2022 and 31st March 2023.

Appropriate transfusion was assessed by the auditor(s) and defined as one that was in keeping with currently guidelines (National Blood Authority Patient Blood Management guidelines, Update consensus guidelines of Warfarin reversal, Evolve 5 recommendations).

Descriptive and comparative analyses was performed using SAS Studio version 9.4.

Results: Nationally, 935 FFP transfusion episodes were entered. 79% of entries were from the public sector and 63% from major city hospitals.

FFP was prescribed appropriately in 21% of cases undergoing therapeutic plasma exchange, 46% of patients requiring warfarin reversal, 22-38% of cases prior to an interventional radiology procedure, 34-62% of cases prior to surgery, 2% of chronic liver disease patients, 3% of patients with disseminated intravascular coagulopathy, 48% of bleeding patients, 7% of patients with a coagulopathy and by convention all cases of massive haemorrhage. Adverse events were seen in 2% of patients including 2 cases (0.02%) of anaphylaxis.

There were no statistically significant variables associated with appropriate FFP use.

Conclusion: This is the 1st Australian study assessing the use of FFP at a national level and has highlighted the widespread misuse of the product. Part of the challenge in assessing the appropriate use is a lack of clear guidance to direct transfusion decisions.
These findings highlight the need for a collaborative approach to optimising FFP use in Australia and provide support for the development of robust national guidelines.

Does transfusion improve quality of life in patients with MDS?

Mo A

Anaemia is common in patients with myelodysplastic syndromes (MDS), and is associated with poorer quality of life (QoL). Transfusion is a cornerstone of supportive care management in MDS patients. However, does transfusion actually improve QoL in patients with MDS?

In this session, QoL assessment tools and methodology will be explored. Results from a recently conducted systematic review investigating whether anaemia treatments improve QoL in MDS patients and other recent literature will also be discussed.

Transfusion associated graft versus disease

Crispin P

Transfusion associates graft versus host disease (TA-GVHD) is a rare complication of transfusion caused by engraftment and tissue infiltration by donor derived T-cells in recipients. Rash, fever, bone marrow failure and liver disease are commonly seen. T-cell proliferation is advanced by the time of presentation and it is almost universally fatal, creating a strong incentive to prevent it. TA-GVHD is driven by three key factors: HLA similarity between donor and recipient; recipient T-cell immunocompromise; and immunoreactive T-cell numbers within the transfused product. Traditional risk mitigation has targeted irradiation of cellular products for immunocompromised patients and those likely to receive HLA matched products. There is increasing understanding that of product-related factors contribute to TA-GVHD. The age of blood is inversely associated with TA-GVHD risk. T-cells become less responsive during cold storage, with similar reactivity as irradiated cells at 21 days and no known cases of TA-GVHD after 14 days of storage. Similarly, by reducing T-cell numbers, pre-storage leukodepletion reduces, but does not eliminate, TA-GVHD. Risk assessment should include consideration of donor and product factors when deciding on prevention strategies.

Iron refractory iron deficiency anemia: Pathophysiology and clinical management of a frequently missed iron disorder

Swinkels D, Sanquin Blood Bank, Amsterdam and Radboud university medical center, Nijmegen, the Netherlands

Patients with Iron Refractory Iron Deficiency Anemia (IRIDA) often present in childhood with fatigue and iron deficiency anemia. IRIDA is a rare autosomal recessive anemia. The disease is a frequently missed diagnosis. It is caused by mutations in the *TMPRSS6* gene encoding Matriptase-2 (MT-2). MT-2 is a liver transmembrane serine protease that plays an essential role in down-regulating hepcidin, the key regulator of iron homeostasis. Hepcidin decreases blood iron concentrations by limiting dietary iron absorption and iron release from stores.

To date, around 90 different *TMPRSS6* variants have been identified in approximately 120 patients of 90 families worldwide, all spread along the MT-2 large ectodomain. Hallmarks of IRIDA are inappropriately high hepcidin levels that result in remarkably low transferrin iron saturation (TSAT) and low/normal serum ferritin and as a consequence, microcytic hypochromic anemia. Current treatment consists of oral iron administration combined with vitamin C or intravenous iron administration, that lead to partial and incomplete correction of anemia. Many but not all IRIDA patients are refractory to oral iron.

The mechanism of hepcidin inhibition by MT-2 is not completely clear. *In vitro* studies indicate that activated MT-2 cleaves multiple components of the hepcidin induction pathway. Recent *in vivo* studies in mice, however, suggest that MT- 2 merely inhibits (basal) hepcidin expression by a non -proteolytic pathway, i.e. binding to and interfering with the hepcidin binding complex including hemojuvelin and TFR2. These data suggest that this inhibition is lacking in IRIDA patients with pathogenic MT-2 variants, resulting in elevated basal hepcidin levels.

In the absence of inflammation, a decreased TSAT/hepcidin ratio has been reported as promising diagnostic tool for IRIDA. However, implementation of this ratio in the clinic requires the definition of its gender and age specific clinical decision limits, preferably defined by a standardized hepcidin assay. In the diagnosis of IRIDA several other questions remain. First of all, of some newly found missense variants in *TMPRSS6,* the pathogenicity remains unclear, necessitating functional tests to prove it is the disease causing variant. In this context, identification of MT-2 substrate/s is mandatory. Second, although IRIDA has been described as an autosomal recessive disorder, several phenotypically affected patients have been described for whom only a heterozygous TMPRSS6 variant was found. Alternatively causes of the underlying pathophysiology of the IRIDA phenotype in these patients include the presence of non-coding TMPRSS6 variants, polygenetic inheritance, and modulating environmental factors.

At the end of my presentation you will be able to better diagnose and treat TMPRSS6-related IRIDA patients among your patients with iron deficiency anemia.

How to diagnose iron dyshomeostasis: Perspective of the clinical biochemistry lab

Swinkels D, Sanquin Blood Bank, Amsterdam and Radboud university medical center, Nijmegen, the Netherlands

The diagnosis of iron disorders can be complex. Many different biochemical and hematological parameters are available for the diagnosis of iron dyshomeostasis. Each of these parameters informs us on a specific part of iron metabolism or a combination thereof, i.e. iron stores (ferritin), (functional) iron availability for erythropoiesis (transferrin bound iron, sTfR, ZnPP, Hb, and Hb-indices), status of the regulatory axis for systemic iron metabolism (hepcidin) and its communication with erythropoiesis (erythroferrone). Moreover, molecular pathways for iron and inflammation highly interact. Hence inflammation status needs to be accounted for when interpreting iron parameters in a patient. Therefore in determining body iron status it is important to consider the whole picture rather than relying on single tests.

Interpretation and definition of reference intervals, common decision limits, and development of guidelines is further complicated by lack of standardization and harmonisation of many parameters, among which are ferritin, sTfR, ZnPP, reticulocyte – Hb content parameters, hepcidin and erythroferrone. Lack of awareness of the resulting between laboratory differences in measurements obtained by different assays has resulted in differences in conclusions from literature and diagnostic strategies advocated in the various guidelines. Measurement results are also affected by pre-analytical handling of samples and interference of treatments (i.e. IV iron, chelator) in the chemical reaction underlying the assay.

At the end of my presentation you will be able to interpret the different iron parameters in the context of pathways of iron metabolism in health and disease. You will be aware of the effect of sample handling, treatment interferences and lack of standardization and harmonisation on many iron parameters and its consequences for the diagnosis of iron disorders. Finally, in your daily practice (and in a quiz at the end of my presentation), you will be able to differentiate the various iron disorders by using the measurement results of a combination of iron parameters.

New insights into the pathogenesis and prevention of TRALI

Tung J

Adverse transfusion reactions are increasingly rare. In some cases, patients develop life-threatening difficulty in breathing either during or soon after a transfusion. This can be due to transfusion-related acute lung injury (TRALI) or transfusion-associated circulatory overload (TACO). Both are defined clinically by the acute onset of hypoxaemia and pulmonary oedema, but are distinguished by the presence of left atrial hypertension in TACO but no TRALI.

Many cases of TRALI are caused by the transfusion of alloantibodies against leucocyte antigens – either human leucocyte antigens (HLA) or human neutrophil antigens (HNA). These alloantibodies typically develop as a result of pregnancy or transfusion. Hence to reduce the risk of TRALI, many blood providers limit use of plasma and plasma-containing components from female donors. In Australia, the switch to male donors for clinical plasma and aphaeresis platelets, and the use of platelet additive solution to reduce the plasma content of both aphaeresis and pooled platelets, has reduced the incidence of TRALI. However, cases of TRALI continue to occur. Many of these residual cases of TRALI are likely related to the accumulation of bioactive mediators in blood components during routine storage. These mediators, commonly referred to collectively as biological response modifiers (BRMs), include various proteins, lipids, and extracellular vesicles.

The precise cellular pathways by which TRALI develops remain uncertain. Evidence from experimental and animal models suggests that at least six different pathways are involved in TRALI pathogenesis. The six pathways appear to converge upon the release of reactive oxygen species that, in the absence of a microbial target, instead damage the pulmonary endothelium, resulting in fluid leakage and TRALI development. Three of these pathways are dependent upon neutrophils, while the others are not. The pathways also differ in the cell targeted by the antibody or BRM, as well as the effector cell responsible for releasing the ROS. There is also evidence that neutrophil extracellular traps (NETs) play a role. This presentation will describe the six pathways, as well as the evidence supporting them. A better understanding of how TRALI develops is crucial to the design of new strategies to prevent TRALI and improve transfusion safety.

There are no specific treatments for TRALI. Clinical support involves providing the patient with supplemental oxygen, and in severe cases, mechanical ventilation. There is evidence from experimental and animal models supporting some novel treatments (e.g. ROS inhibitors, IL-10, DNase) for TRALI. This presentation will also review this evidence.

The immune characteristics and function of refrigerated and cryopreserved platelet components

Winskel-Wood B

Platelets are highly dynamic cells that can rapidly respond to activation signals. Platelet activation causes significant changes in cellular structure and function. This activity is commonly associated with platelet haemostatic function; however, platelets also play a substantial role as modulators of the immune system. In general, platelet immune function is beneficial; however, dysregulation is linked to the pathogenesis of inflammatory disorders and the risk of adverse events post-transfusion. While the cause of adverse events is multifactorial, platelet immune activation caused by the manufacture and storage of platelet components is believed to contribute.

Platelet components are stored at room temperature, limiting the shelf-life to 7 days due to the risk of bacterial proliferation and a gradual reduction in haemostatic function. Consequently, there has been renewed interest in the clinical evaluation of alternative platelet storage methodologies, including refrigeration and cryopreservation. However, little is known about how these storage modes affect the immune characteristics and function of platelets. Given the link between platelet immune function and adverse events, this is critical knowledge. As such, this presentation aims to explore how refrigeration and cryopreservation affect the immune characteristics and function of platelets.

Novel mechanisms of thrombopoiesis

Gardiner E

Platelets play a crucial role in maintaining haemostasis, and dramatically reduced levels of circulating platelets (thrombocytopenia) elevates bleeding potential and interferes with innate immune responses. Thrombocytopenia is a comorbidity that frequently occurs in disease and as a side effect of clinical therapies, most notably in cancer chemotherapy. Treatments that directly increase platelet count are limited to thrombopoietin (TPO) receptor agonists and platelet transfusion which carry potential side effects and are not without risk.

Work to be described here explores the cellular and molecular mechanisms underlying a newly identified thrombopoietic property of CX-5461, a first-generation inhibitor of ribosomal biogenesis that ablates RNA polymerase I (Pol I)-mediated transcription of ribosomal DNA. CX-5461 is being evaluated as an anticancer therapeutic, as ribosomal biogenesis is intrinsically linked to cell proliferation and processes frequently upregulated during tumorigenesis. This work provides the first evidence of a drug-induced TPO-independent MK-biased haematopoiesis leading to enhanced circulating platelet levels in mice and in humans.

Mechanobiology inspired anti-thrombotic strategies and point-of-care microtechnologies

Ju L

Affiliation: School of Biomedical Engineering, Faculty of Engineering, Charles Perkins Centre, Heart Research Institute and Sydney Nano Institute, The University of Sydney;

Abstract:

Dr. Ju's work in mechanobiology connects mechanical forces in blood flow to hematological proteins and blood clotting cells, offering innovative solutions for blood clotting diseases. The Biomembrane Force Probe (BFP), a nano-tool invented by Dr. Ju, enables precise, quantitative mechanobiology investigations at single-cellular to molecular scales.

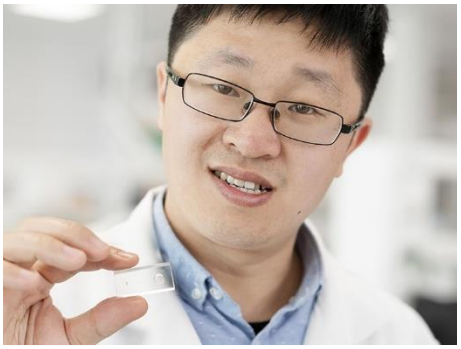
In collaboration with the Charles Perkins Centre and Royal Prince Alfred Hospital, Dr. Ju leads a team developing microfluidic devices for personalized diagnostics and telehealth. Two novel platforms have been created to study thrombosis and cancer mechanobiology. The microvasculature-on-a-post chip mimics partially stenotic vascular geometries, using a 3D micropost structure and endothelial cells to investigate the impact of disturbed flow on thrombotic response. The Vein-Chip recapitulates cerebral venous sinus thrombosis (CVST) vascular anatomy, enabling systematic characterization of venous thrombogenesis concerning fibrin formation and platelet aggregation.

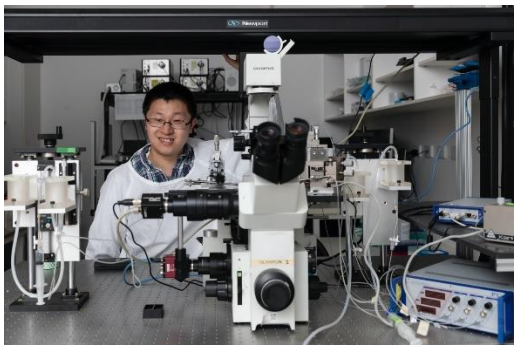
These platforms provide a controlled, patient-specific environment for personalized evaluation of prothrombotic phenotypes and the risk of heart attack, stroke, and COVID-19 vaccine-induced immune thrombotic thrombocytopenia. The integration of microfluidics and vascular biology holds potential for targeted therapeutics development, revolutionizing thrombosis research and personalized diagnostics.

My Short Bio:
Associate Professor Arnold Ju received his PhD in Biomedical Engineering at Georgia Institute of Technology and Emory University, USA. In 2014, he joined the Australian Centre for Blood Diseases, Monash University, Melbourne as a junior postdoc; and relocated in 2015 to Sydney, to join the Heart Research Institute. In early 2020, Dr Ju joined the University of Sydney (USYD)'s new BME school as a senior lecturer and started up the Mechanobiology and Biomechanics Laboratory (MBL).

A/Prof Ju currently holds an Heart Foundation Future Leader Fellowship, working at the interface between mechanical engineering and mechanobiology. His team has pioneered multiple biomechanical nanotools, including blood clot-on-chip microfluidic devices (***Nature Materials*** 2019), single-cell biomembrane force probes (***Nature Communications*** 2018), and 4-D haemodynamic modelling (***Nature*** 2021). Recently, he was awarded the prestigious mid-career Snow Fellowship.

His vision is to build novel platforms that integrate advanced biomanufacturing, high-throughput biomechanical manipulation, and artificial intelligence for biobank data processing. His track record spans developing, characterising, and evaluating innovations of 3D organoids and organ-on-chips, mechanobiology, imaging probes and biosensors, bio-nanotechnology, and image-based deep learning. These large facilities should provide significant benefits to interdisciplinary research in biofabrication, biomechanics and point-of-care microtechnologies.





Microfluidic applications in immunothrombosis

Passam F

Immunothrombosis in Haematology includes thrombotic disorders caused by activating antibodies such as heparin induced thrombocytopenia (HIT) or antiphospholipid syndrome. A 3rd entity emerged during the COVID-19 vaccination rollout which was vaccine induced thrombotic thrombocytopenia (VITT). Apart from platelet activation, these conditions are accompanied by vascular damage and inflammation. There are limited tests to directly measure vascular damage or inflammation in immunothrombotic diseases. In this presentation, I will discuss the applications of a microfluidic endothelialized device, we have named Endo-chip, for the study and measurement of vascular damage by autoantibodies in immunothrombosis.

Idiopathic upper extremity deep vein thrombosis

Yuen H

Idiopathic upper extremity deep vein thrombosis (IUEDVT) is an uncommon condition which is debilitating for affected individuals due to post thrombotic syndrome (PTS) and treatment morbidity. During this session we will review the evidence behind current treatments for UEDVT, diagnostic strategies for venous thoracic outlet syndrome (VTOS) and outcomes for UEDVT in terms of recurrent thrombosis and post thrombotic syndrome (PTS).

The main treatment modalities include anticoagulation or anticoagulation together with additional interventions such as thrombolysis, angioplasty and decompressive surgery aiming to reduce the risk of recurrent thrombosis or PTS. Currently there are no head-to-head studies comparing these strategies and there are only low grade, single-arm studies which are at moderate to serious risk of bias.

Surgical intervention aims to rectify VTOS which is described as compression of the axillosubclavian vein upon arm abduction. However, there is a lack of gold standard diagnostic imaging study for VTOS. Although dynamic upper limb ultrasonography (DULUS) is often utilised, methodology and reference ranges have not been standardised. Other modalities used to date for VTOS diagnosis include CT or MRI however evidence behind this practice is again scant. Long term treatment outcomes are unclear from the literature and further research is required to delineate rates of PTS and recurrent thrombosis post treatment for IUEDVT and ultimately guide treatment decisions.

The evolution of the genetic haematology service at Peter MacCallum Cancer Centre and Royal Melbourne Hospital

Fox L

The bone marrow failure syndromes (BMFS) are clinically diverse, with both inherited and acquired etiologies. Deleterious germline variants conferring a susceptibility to bone marrow failure (BMF) or hereditary haematological malignancy (HHM) are increasingly recognised. Alongside the rapid developments in this field, we have established a Genetic Haematology clinic dedicated to comprehensive care of patients with BMF/HHM, with a focus on achieving accurate diagnosis and permitting participation in research. Our initial BMF/HHM project was the Melbourne Genomics Health Alliance Bone Marrow Failure Flagship, which aimed to improve diagnostic accuracy utilising targeted sequencing and whole exome sequencing in 115 patients with BMF.[1] Along with utility of testing, it was demonstrated that patients with BMF often present unique, complex management issues and frequently experience poor outcomes due to multiple factors, including the rarity of the individual conditions. These observations lead to establishment of the Evaluating Multidisciplinary Bone maRrow fAilure CarE (EMBRACE) study - a multi-stage hybrid implementation-effectiveness study that aimed to address challenges by first understanding the nature and scale of issues faced by these patients and their physicians, and then developing, implementing, and evaluating a comprehensive 10-component model of care (MoC10). The MoC10 has been applied to 455 patients and at-risk relatives as part of the Genomic Haematology clinic and through the genomic testing arm of the study/model of care, 310 patients with BMF have received comprehensive genetic testing with causative germline mutations identified in 37 patients (12%). Our current diagnostic study is the Medical Research Future Fund sponsored IBMDx study which aims to achieve diagnosis, discovery and novel phenotype characterisation using multimodal genomics (whole genome transcriptome sequencing) in patients with inherited bone marrow failure and related disorders. Variants of interest have been presented at national monthly variant review meetings (37 meetings have occurred to date). These virtual meetings are attended by between 60-100 clinicians, scientists, and researchers nationally which now serves as a national focal point for health professionals interested in BMF/HHM.

[1]Utility of clinical comprehensive genomic characterisation for diagnostic categorisation in patients presenting with hypocellular bone marrow failure syndromes. Haematologica. Blombery P, Fox LC, Ryland GL, Ritchie D et al. Haematologica 2021 Jan 1;106(1):64-73

Case study: Glycosorb-ABOi secondary plasma device procedure

Jeans R

The Glycosorb-ABOi column procedure is the easiest apheresis procedure we do, so what could possibly go wrong?
Glycosorb®-ABO column apheresis reduces serum anti-A and anti-B titres, facilitating blood group-incompatible kidney transplantation from living donors. This is a case study review of a complex and dynamic procedure for a young man having a blood group incompatible renal transplant from his mother. A number of unusual complications occurred simultaneously and this review looks at what was done at the time to troubleshoot, utilising multidisciplinary resources, and what could be done in the future to prevent or reduce these complications from occurring. This was a stressful procedure for both the patient and apheresis staff, but ultimately ended with a positive outcome for the patient. It specifically highlights the need for high level communication and problem solving skills, and the value and importance of experienced apheresis nurses.
Interaction and feedback from the audience will be welcomed during this presentation.

Romony Jeans | Clinical Nurse Educator – Cancer Centre
RN BSc (Nursing), Dip Cancer Nursing
Nursing and Midwifery Education Service
South Metropolitan Health Service
Fiona Stanley Fremantle Hospitals Group
Level 2 Education Building, 11 Robin Warren Drive, MURDOCH WA 6150
Postal address: Locked Bag 100, PALMYRA DC WA 6961
T: 6167 2851 | P: 72851
E: romony.jeans@health.wa.gov.au

Care Plus: a study exploring early intervention with palliative care

Bellingham K - The University of Melbourne & St. Vincent's Hospital Melbourne, Philip J **-** The University of Melbourne & St Vincent's Hospital, Rizvi F **-** The University of Melbourne & St. Vincent's Hospital, Chua J **-** The University of Melbourne & Peter MacCallum Cancer Centre

Aim:

To explore the strategies and challenges of implementing an early palliative care (PC) pathway called 'Care Plus' for patients with Multiple Myeloma (MM).

Method:

As part of a stepped-wedge mixed methods implementation trial testing usual care (control) versus early PC integration (Care Plus) as a practice change across cancer services at four Australian metropolitan hospitals for cancer patients including MM. All MM patients reaching nominated trigger points (time of diagnosis or relapse) were automatically offered Care Plus described as an 'extra layer of care' with specialist PC services, alongside their usual cancer care. Qualitative semi-structured interviews with MM and palliative clinicians, and MM patients were conducted during pre-practice, practice and post-practice change phases of the trial to explore the implementation strategies and acceptability of Care Plus. Interview transcripts were thematically analysed with member-checking to maintain rigour and validity.

Results:

From November 2020 to May 2022, 99 MM patients received Care Plus. Researchers conducted 20 interviews with haematologists (n=4), PC physicians (n=9), clinical nurses (n=3), MM patients (n=4), and MM nurse care coordinators (n=4). Major themes included: the benefits of standardised points for automatic referral to early PC to enable system wide change, development of introductory language resources, enhanced collaborative practice between MM and PC teams, and the value of early PC activities including for asymptomatic patients. A Care Plus toolkit was developed with strategies and resources to implement Care Plus within existing systems.

Conclusion:
Care Plus was notably well received by MM patients, referring haematologists and PC teams and appeared to establish equitable standardised referral processes while fostering collaborative confidence to flexibly address complex care needs of MM patients and carers within a shifting landscape of MM treatment.

The Out with Cancer Study: LGBTQI Cancer Survivorship and Care

Power, R.,* Ussher J.M, Allison, K.A. and Perz, J.
on behalf of the Out with Cancer Study Team.
Translational Health Research Institute, Western Sydney University, Australia

Abstract

There is growing acknowledgement of the psycho-social vulnerabilities and health disparities experienced by people with cancer and carers who are lesbian, gay, bisexual, trans, queer and intersex (LGBTQI). This paper provides insight to these issues drawing on a multi-method Australian Research Council Linkage funded research project, involving surveys, semi-structured interviews and photo-elicitation. 430 LGBTQI patients, representing a range of tumor types, sexual and gender identities, and age groups, 131 LGBTQI carers, and 357 oncology healthcare professionals took part in the study. We also report on our review of LGBTQI inclusiveness of 61 Australian cancer information websites.

LGBTQI people with cancer and their carers reported rates of distress 3 to 6 times higher than the broader cancer population. Difficulties navigating cancer or caregiving whilst also managing additional minority stressors linked to being LGBTQI were reported including experiences of discrimination, rejection from family, isolation from LGBTQI communities, invisibilization and cis-heteronormativity in cancer care and support services. Disclosure of identity was a major cause of distress for LGBTQI cancer patients, with less than 1 in 5 'out' to all healthcare professionals involved in their cancer care. Oncology healthcare professionals reported lack of knowledge and confidence treating LGBTQI cancer patients and wanted more education. Only 13% of Australian cancer organizations mentioned LGBTQI people on their websites; cancer information often made cis-heteronormative assumptions that excluded LGBTQI people.

Building oncology HCP communicative competence to work with LGBTQI patients needs to become an essential part of basic training and ongoing professional development. Visible indicators of LGBTQI inclusivity are essential, alongside targeted resources and information for LGBTQI people.

References

1. Ussher, J. M., Power, R., Perz, J., Hawkey, A. J., & Allison, K. (2022). LGBTQI Inclusive Cancer Care: A Discourse Analytic Study of Health Care Professional, Patient and Carer Perspectives. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.832657
2. Ussher, J. M., Perz, J., Allison, K., Power, R., Hawkey, A., Dowsett, G. W., Hickey, M., Parton, C., McDonald, F. E. J., Davis, I. D., Quinn, G. P., Boydell, K., Robinson, K. H., Chambers, S., & Anazodo, A. (2022). Attitudes, knowledge and practice behaviours of oncology health care professionals towards lesbian, gay, bisexual, transgender, queer and intersex (LGBTQI) patients and their carers: A mixed-methods study. Patient Education and Counseling, 105(7), 2512-2523. https://doi.org/10.1016/j.pec.2021.12.008
3. Power, R., Ussher, J. M., Perz, J., Allison, K., Hawkey, A. J., & Team., T. O. w. C. S. (2022). "Surviving discrimination by pulling together": LGBTQI cancer patient and carer experiences of minority stress and social support. Frontiers in Oncology, 12, 918016. https://doi.org/10.3389/fonc.2022.918016
4. Ussher, J. M., Allison, K., Perz, J., Power, R., & The Out with Cancer Study Team. (2022). LGBTQI Cancer Patients' Quality of Life and Distress: A Comparison by Gender, Sexuality, Age, Cancer Type and geographical remoteness. Frontiers in Oncology, 12:873642. https://doi.org/10.3389/fonc.2022.873642
5. Ussher, J. M., Ryan, S., Power, R., Perz, J. & The Out with Cancer Study Team. (2023) Almost invisible: A review of inclusion of LGBTQI people with cancer in online patient information resources. Patient Education and Counseling, 114, 107846 https://doi.org/10.1016/j.pec.2023.107846
6. Ussher, J.M., Power, R., Allison, K., Sperring, S., Parton, C., Perz, J., Davies, C., Cook, T., Hawkey, A.J., Robinson, K.H., Hickey, M. Reinforcing or disrupting gender affirmation: the impact of cancer on transgender embodiment and identity. Archives of Sexual Behavior. 2023 Apr;52(3):901-20. https://doi.org/10.1007/s10508-023-02530-9

Wogan A

Burnout and attrition are two of the biggest challenges facing nurses and midwives at the current time. Executive Coaching, routinely offered to business leaders, is rarely offered to nurses and midwives throughout their careers. Research shows that through coaching, nurse managers gain increased resilience, confidence, and better coping mechanisms (Wescott, 2016). Executive coaching for nurses and midwives is now an aspiration of the WHO and the ICN.

Society recognises that we need to keep nurses in the health system and support them to stay and be eaders and influential in the health system as well as supporting those in more senior leadership positions.

This interactive leadership workshop will provide nurses with

- A significant growth self-awareness, emotional intelligence, and confidence and ability to influence more effectively
- Helping nurses be the best leader they can be, intentional leaders
- Builds confidence and healthy growth mindset
- Encourages new ways of thinking and responding
- Improves the effectiveness of communication

Healthcare professionals who know themselves better than they have before

- Achieve better health outcomes for their patients
- Have more effective sustainable careers
- Create better reputations for the organisations they work for
- Have less absenteeism and less turnover and
- Generate more team cohesion at work.
- Lead their teams more effectively and team health and performance will demonstrably chance for the better

The world we now live in is more visual than oral, people remember what they see.
I will be equipping you with immediately applicable, visual leadership frameworks and strategies that are used as mirrors to enhance self-awareness, performance, and trust.

Visual leadership tools allow your people to carry with them the type of leadership you would like them to model and multiply, to build a leadership culture and a leadership model that is inclusive that everyone can buy into.

From bench to bedside - nuances of collection and delivery for the various CAR-T's delivered at PMCC

Davawala J

Peter MacCallum Cancer Centre (PMCC) has an established program for delivering CAR-T and other Cellular immunotherapy products to patients as part of standard of care and clinical trial programs. Since 2019, 133 patients have received CAR-T immunotherapy as standard of care at PMCC. This presentation will provide insight into day-to-day challenges faced in managing end-to-end workflows for both standard of care and clinical trials while highlighting differences in workflows for allogeneic and autologous CAR-T products. The challenges faced in scaling up existing processes with the projected rapid increase in the volume of CAR-T therapies delivered as standard of care will be explored.

Bench to bedside (impact of COVID - pre and post COVID data assessing fresh vs cryopreserved products)

Swain M

At the Alfred hospital the cryopreservation of autologous haematopoietic stem cells and cord blood prior to thaw and infusion has been standard of care for over 20 years. Whilst it had been standard for care to infuse allogenic haematopoietic stem cells fresh. This changed on 05-May-2020 when the Alfred receipted a cryopreserved international unrelated allogenic haematopoietic stem cell product that had been cryopreserved at a German cryopreservation hub and transported in a dry shipper to the Alfred hospital.

With an increase in the number of COVID-19 cases within Australia and around the world there was a rapid rise in both the uncertainties of patient and donor availability due to COVID-19 exposure and the logistics in transporting fresh HPC(A)s into and around Australia. However, allogeneic hematopoietic stem cell transplantations could not be delayed for patients with haematological malignancies, especially acute leukemias.

As a result, imported allogeneic donations were cryopreserved at the collection centre and transported to the transplant centre for cryogenic storage prior to thaw for infusion. The patient's pre transplant conditioning was delayed until:

- Receipt of the cryopreserved product at the transplant centre

1. Evidence of cryogenic temperature maintenance of the product during shipment
2. Satisfactory Cryo QC Testing – performed on sentinel vial cryopreserved at the time of product cryopreservation. The vial was shipped with the cryopreserved product and the thawed viable CD34 recovery and CD34 viability (%) was determined by the Alfred Health Flow Cytometry laboratory.

   Target test results: a) Viable CD34 recovery ≥ 57%*

   b) % Viable CD34 cells ≥ 64%*

   *The target results were based on the cryopreserved autologous HPC(A) release criteria.

We retrospectively compared the cryopreserved allogeneic transplant neutrophil and platelet engraftment times from 05-May-2020 up to 31-Dec-2021 with the fresh allogeneic transplant neutrophil and platelet engraftment times from 01-Jan-2020 to 31-Dec-2021 as well as the previous 9 years. This analysis was performed to determine the suitability of the autologous CryoQC release testing criteria for cryopreserved allogeneic HPCs to be considered standard of care.

Monitoring transfusion and patient safety risks

O'Beid P[1]
[1]*Nsw Health Clinical Excellence Commission, St Leonards, Australia*

Aim: To understand the incidence of avoidable transfusion practice incidents, to inform transfusion and patient safety improvement priorities.

Method: An observational study based on retrospective analysis of patient incident notifications in NSW public facilities. Incidents for inclusion were identified within and, extracted from the NSW Health incident management system (*IIMS and ims+)* databases by notifications submitted:

- July 2016 – June 2020
- Incident type of 'Blood and Blood products'
- Under other incident types, where the key word of transfusion is included in the notification.

Incidents were assigned to specific transfusion patient safety incident categories, to enable analysis.

Results: In total, 9537 notifications were analysed. 18% (n=1690) were categorised as transfusion related complications, and 82% (n=7834) were categorised as incidents that were unrelated to transfusion complications.

74% (n=5796) of notifications classified as incidents related to labelling and identification and wastage. 26% (n=2038) related to other incident types, including: Wrong Blood In Tube; non-lab dispensing; lab processes; Incorrect Blood Component Transfused; equipment; documentation; clinical management and the administration process.

Analysis of the broader dataset identifies opportunities to improve the incidence of avoidable transfusion practice incidents. During the reported period, two key areas were identified for urgent patient safety improvement. These were related to emergency use uncross matched plasma compatibility and patient identification bands that are covered by sterile drapes. Two NSW Health Safety Information bulletins were issued to the health system.

Discussion/Conclusion: Haemovigilance as described by the World Health Organisation, includes preventing unwanted transfusion related events covering all activities in the transfusion chain. The collection, analysis and surveillance of incident data related to all adverse events associated with transfusion practice is integral to understanding the full range of blood management patient safety risks. Conclusions drawn from these data contribute to understanding transfusion patient safety priorities to inform improvement initiatives and improve patient outcomes in transfusion.

Characterisation of the Kidd blood group system in a Kenyan Population

Githiomi R[1], Perros A[3], Sowah S[3], Obiero C[4], Lopez G[3], Roulis E[3], Flower R[3], Hyland C[3]
[1]Department of Health Policy and Research, Ministry of Health, , Kenya, [2]Department of Medical Laboratory Sciences, Medical School, Mount Kenya University , Thika, Kenya, [3]Research & Development, Australian Red Cross Lifeblood, Brisbane, Australia, Brisbane, Australia, [4]Tissue and Transplant Authority Kenya (formally Kenya National Blood Transfusion Service), Nairobi, Kenya, [5]Faculty of Health, Queensland University of Technology, Brisbane, Australia, Brisben, Australia

ANZSBT: Patient Blood/Management; No conflict of interest to disclose

Characterisation of the Kidd blood group system in a Kenyan population

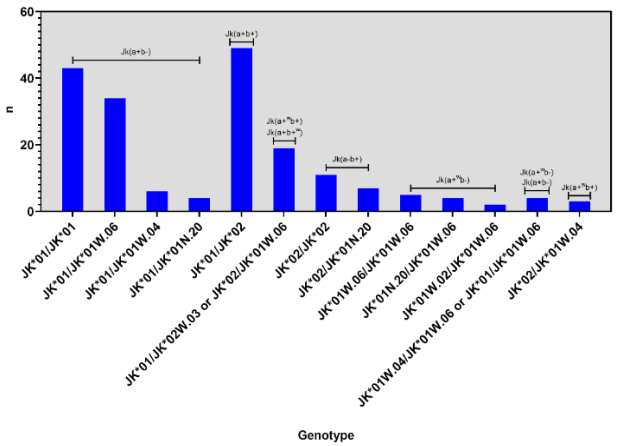
Aim: In Africa there are limited reports on the phenotype distribution for the 44 blood group systems recognised by the ISBT. In a collaborative study with the Kenya National Blood Transfusion Service (KNBTS) we recently reported the distribution for Duffy and Kell variants; this report concerns distribution of variants in the *SLC14A1* gene – the Kidd (JK) system.

Method: DNA was extracted from blood from 191 KNBTS donors. Next generation sequencing was performed at Lifeblood using a targeted custom blood group sequencing panel on an Illumina MiSeq[9]. JK genotype and predicted phenotype were determined independently by two research scientists using variant calling format files and ISBT Blood Group Allele Tables to provide blood group genotype and predicted phenotype.

Results: Thirteen JK genotype combinations were detected in this cohort (Figure 1). Predicted phenotype distribution of the Kidd blood group system was as follows: Jk(a+b-) 45.5%; Jk(a+b+) 25.7%; Jk(a+^{w}b+) or Jk(a+b+^{w}) 9.9%; Jk(a-b+) 9.4%; Jk(a+^{w}b-) 5.8%; Jk(a+^{w}b-) or Jk(a+b-) 2.1%; Jk(a+^{w}b+) 1.6%.

Conclusion: In our cohort, the Kidd phenotype distribution pattern was consistent with that reported elsewhere in Africa of Jk(a+b-)>Jk(a+b+)>Jk(a-b+)[1, 2]. This frequency pattern is also observed in Sub-Saharan Africa including Ghana, Mali, Nigeria, Burkina Faso[2,3,4] and an Egyptian population[2,5]. Surprisingly 3 genotypes were associated with weak Kidd antigen expression and such samples can be missed by serological typing. Variants encoding Kidd alleles had similar frequencies to reports for the African population, however variants for weak alleles JK*01W.02 and JK*01W.03 had lower frequencies. No null variants were detected, as expected for an African population[1]. The study provides the basis for further research into tools and infrasctures that can be used for extended red cell serological and genotyping in the KNBTS, to improve transfusion practices and develop a genomic reference library.

Figure 1. Kidd blood group system genotype combinations



References

- JR Mamilton. Kidd blood group system: a review. Immunohematology. 2015;31(1):29-35

1. Vorholt et al. High-Throughput Next-Generation Sequencing of the Kidd Blood Group: Unexpected Antigen Expression Properties of Four Alleles and Detection of Novel Variants. Transfus Med Hemother 2023;50:51–65.
2. Boateng et al. Red blood cell alloimmunization and minor red blood cell antigen phenotypes in transfused Ghanaian patients with sickle cell disease. Transfus. 2019;59;2016–2022
3. Ba A, Bagayoko S, Chiaroni J, Baiily P, Silvy M. Genotyping of 28 blood group alleles in blood donors from Mali: prediction of rare phenotypes. Transfus Apher Sci. 2016 Apr;54(2): 289–95
4. Randa M. Abou El-Fetouh, et al. Prevalence of Red Cell Blood Group Antigens Among Egyptian Population. Med. J. Cairo Univ., Vol. 86, No. 2, March: 989-994, 2018
5. Acquaye JK. Red cell antigens in the Ewe ethnic group of Ghana. Ghana Med J 1992;26:438-47.
6. Acquaye JK. Red cell antigens in the Ga ethnic group of Ghana. Ghana Med J 1990;24:177-82.
7. Erhabor O, Hassan M, Alhaji YB, et al. Kidd blood group phenotypes among pregnant women in Sokoto, North Western Nigeria. Asian Pac J Trop Med 2014;7(Suppl 1):S111-5.
8. Eileen Roulis et al. Translation of a customised blood sequencing panel to the reference laboratory , DOI: https://doi.org/10.1016/j.pathol.2021.12.238

Investigating biomarkers of bleeding in neonates and infants undergoing cardiac surgery with cardiopulmonary bypass using proteomics

Van Den Helm S[1,2], Davies P[1,2,3], Emery-Corbin S[4,5], Yousef J[4], Morrison S[3,6], Konstantinov I[2,7,8], Davies B[2,7], Horton S[2,9], Ignjatovic V[1,2,10,11], Monagle P[1,2,12,13], Attard C[1,2]

[1]Haematology Research, Murdoch Children's Research Institute, Parkville, Australia, [2]Department of Paediatrics, The University of Melbourne, Melbourne, Australia, [3]Department of Anaesthesia and Pain Management, The Royal Children's Hospital, Melbourne, Australia, [4]Advanced Technology and Biology, Walter and Eliza Hall Institute, Parkville, Australia, [5]Department of Medical Biology, The University of Melbourne, Melbourne, Australia, [6]Department of Anaesthesia and Pain Management, Queensland Children's Hospital, Brisbane, Australia, [7]Department of Cardiac Surgery, The Royal Children's Hospital, Melbourne, Australia, [8]Heart Research, Murdoch Children's Research Institute, Melbourne, Australia, [9]Perfusion Department, The Royal Children's Hospital, Melbourne, Australia, [10]Institute of Clinical and Translational Research, Johns Hopkins All Children's Hospital, St Petersburg, USA, [11]Department of Pediatrics, School of Medicine, Johns Hopkins University, Baltimore, USA, [12]Department of Clinical Haematology, The Royal Children's Hospital, Melbourne, Australia, [13]Kids Cancer Centre, Sydenye Children's Hospital, Randwick, Australia

Aim: Each year in Australia, approximately 3000 babies are born with congenital heart disease (CHD). Surgery for CHD frequently involves the use of cardiopulmonary bypass (CPB). Bleeding is a significant complication in babies that undergo surgery with CPB and is associated with increased risk of death. We aimed to identify biomarkers of bleeding in neonates and infants undergoing surgery for CHD with CPB.

Method: This study was approved by the Royal Children's Hospital Human Research Ethics Committee. Parents/guardians provided written informed consent. Blood samples from babies (<1 year) undergoing CPB were collected at baseline (pre-CPB), pre-protamine (post-CPB), post-protamine, hourly post-protamine until leaving theatre, upon return to the paediatric intensive care unit (PICU) and the next morning in PICU. A pilot proteomic study using data-independent acquisition mass spectrometry (DIA-MS) was conducted on citrated plasma samples from 6 patients (out of 138 recruited in total), 3 who bled post-CPB ("bleeders" defined as requiring recombinant factor VII or prothrombin complex administration) and 3 who did not bleed ("non-bleeders").

Results: A total of 426 proteins were reproducibly identified in the 6 pilot patients. The protein profile changes over time in all patients and bleeders and non-bleeders cluster differently at most time points apart from pre-protamine (Figure 1). A non-redundant total of 129 proteins were differentially abundant between bleeders and non-bleeders at each time point and 175 significant proteins were identified in time course analysis as different between the groups.

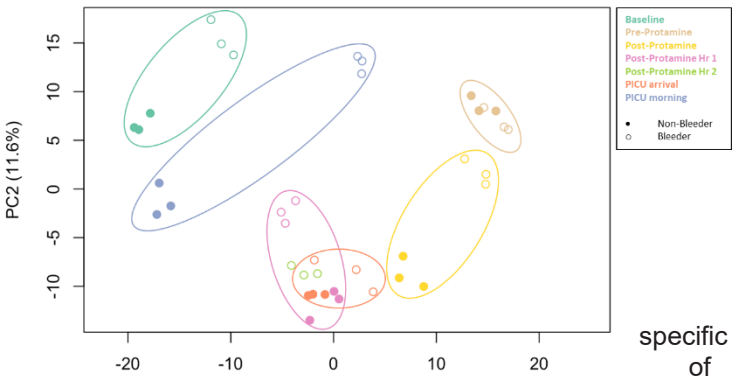


Figure 1. Principal Component Analysis (PCA) plot of the protein profiles of 'bleeders' vs 'non-bleeders'.

Conclusion: This is the first study using discovery proteomics to determine blood markers associated with bleeding. A number proteins are associated with bleeding and further analysis of more patients is required. Differences in protein profiles of bleeders and non-bleeders already exist at baseline and could allow for early identification and prophylaxis of patients with increased risk of bleeding.

specific of

Massively Parallel Sequencing: the next generation non-invasive prenatal testing platform for alloimmunised pregnant women

Lopez G[1,2,7], Sarri M[1,7], McGowan E[1], O'Brien H[3], Daly J[4], Gardener G[5], Flower R[1,6], Hyland C[1,6]
[1]Research and Development, Australian Red Cross Lifeblood, Brisbane, Australia, [2]School of Health, University of the Sunshine Coast, Sippy Downs, Australia, [3]Red Cell Reference Laboratory, Australian Red Cross Lifeblood, Brisbane, Australia, [4]Pathology Services, Australian Red Cross Lifeblood, Brisbane, Australia, [5]Maternal Fetal Medicine, Mater Mothers' Hospital, Brisbane, Australia, [6]School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Kelvin Grove, Australia, [7]Equal first authors, ,

Aim: Haemolytic disease of the fetus and newborn (HDFN) caused by maternal alloimmunisation against fetal red cell antigens can be life-threatening or lead to life-long disability.[1] Non-invasive prenatal testing (NIPT) uses cell-free DNA (cfDNA) to predict fetal blood group antigen status to guide management of at-risk pregnancies.[1,2]

Current NIPT platforms detect one target in a single assay.[1] Massively Parallel Sequencing (MPS) technology has the capacity to investigate multiple targets simultaneously and can be customised for specific population groups.[3] This study aimed to apply MPS technology for NIPT to detect a panel of clinically significant red cell and platelet antigens.

Method: Antibodies against E/e, C/c, K, Fy^a, Jk^a, Lu^a, M, and HPA-1a antigens were identified in 26 pregnant women (11–34 weeks gestation), seven of which had multiple antibodies. A custom, targeted MPS panel, which included control markers for fetal cfDNA presence, was used to genotype the cfDNA samples.[3] Sequencing data was analysed using a bioinformatics workflow and, where available, outcomes were compared with blood group data obtained by validated methods.

Results: There were 34 fetal antigen predictions (18 antigen-positive and 16 antigen-negative) from 26 cfDNA samples. Ten predictions had confirmatory results, and all were concordant. Internal control markers detected fetal cfDNA in all samples. A case involving multiple antibodies (anti-C, anti-e, and anti-Fy^a) had a predicted fetal phenotype of C+, e+, Fy(a–), which was concordant with the infant cord blood phenotype. For two cases, it was discovered upon analysis that fetal genotyping was incorrectly requested for *FY*A* instead of *JK*A*. Re-analysis of the data predicted that the cases were Jk(a+) and Jk(a–) respectively.

Conclusion: MPS has the advantage to test multiple targets in a single assay as well as allowing retrospective analysis of data for other blood group targets without the need for sample recollection.

References

1. O'Brien H, Hyland C, Schoeman E, Flower R, Daly J, Gardener G. Non-invasive prenatal testing (NIPT) for fetal Kell, Duffy and Rh blood group antigen prediction in alloimmunised pregnant women: power of droplet digital PCR. Br. J. Haematol. 2020;**189**: e90-e4.
2. Hyland CA, Millard GM, O'Brien H, Schoeman EM, Lopez GH, McGowan EC*, et al.* Non-invasive fetal RHD genotyping for RhD negative women stratified into RHD gene deletion or variant groups: comparative accuracy using two blood collection tube types. Pathology 2017;**49**: 757-64.
3. McGowan E, O'Brien H, Sarri M, Lopez G, Daly J, Gardener G*, et al.* Non-invasive prenatal testing for a broad panel of red cell and platelet antigens using a targeted sequencing approach: proof-of-principle. Vox Sang 2022;**117 (Suppl 1)**: 6-271.

Ultra-massive transfusion: Predictors of occurrence and in-hospital mortality from the Australian and New Zealand Massive Transfusion Registry (ANZ-MTR)

Maclean M[1], Wellard C[2], Ashrafi E[2], Haysom H[2], Sparrow R[2], Wood E[2], McQuilten Z[2]
[1]*Austin Health, Melbourne, Australia,* [2]*Transfusion Research Unit, Monash University, Melbourne, Australis*

Aim: To examine incidence, characteristics, predictors of in-hospital mortality and 5y survival of patients in the ANZ-MTR with 'ultra-massive' transfusion (UMT).

Method: The ANZ-MTR captured patients at 29 sites receiving MT, defined as ≥5 units of red blood cells (RBCs) in 4h. UMT was defined as ≥20 units RBCs in 48h (Dzik et al, 2016). Predictors of UMT and in-hospital mortality were modelled using multivariate logistic regression, and survival analysis using Kaplan-Meier method and Cox regression.

Results: Of 9028 patients, 803 (8.9%) received UMT. UMT patients were younger (median age 57 vs 62y for MT; p<0.001). In MT and UMT, males predominated (62.9% and 66.3%, respectively); and context was predominantly trauma (23% and 28.8%) and cardiothoracic surgery (CTS) (20.3% and 21.7%). Median RBC units within 4h were 6 (MT) and 16 (UMT). In UMT, 4h FFP:RBCs ratio (0.4 vs 0.6, p<0.001), 4h cryoprecipitate use (39.9% vs 72.9%, p<0.001), and in-hospital mortality was higher (20.5% vs 44.2%, p<0.001). Independent predictors of UMT (Odds Ratio; 95% CI) were age >60y (0.66; 0.56-0.78), baseline Hb >100g/L (1.31; 1.08-1.59), INR >1.5 (1.56; 1.24-1.96), and APTT >60s (4.49; 3.40-5.61). Predictors of in-hospital mortality included UMT (2.27, 1.83-2.80), age >60y (1.35, 1.14-1.60); Charlson score ≥3 (1.53, 1.23-1.89); and bleeding context, with mortality less likely in liver transplant (0.04, 0.02-0.08) and obstetrics (0.1, 0.04-0.23) and more likely in medical (2.28, 1.36-3.83), vascular surgery (2.16, 1.67-2.79) and trauma (1.33, 1.05-1.68) compared with CTS. Baseline APTT >60s (2.57, 1.95-3.40), INR >1.5 (1.77, 1.41-2.22); platelets <150x10^9/L (1.52,1.23-1.85); and 4h FFP:RBC >1.2 (1.18, 1.01-1.39) were independently associated with in-hospital mortality. Nevertheless, 5y survival following discharge was not significantly different between the groups (HR=0.87 [95%CI 0.64-1.18], p=0.38).

Conclusion: UMT patients are more commonly younger, with baseline coagulopathy, and have higher in-hospital mortality compared with MT. However, UMT is not futile: 55.8% survived to discharge, without significant difference in survival post-discharge between the groups.

Reference: Dzik WS, et al. Survival after ultramassive transfusion: a review of 1360 cases. *Transfusion*. 2015;56(3):558–563.

Local Anticoagulation via Gene Transfer: Towards Prevention of Cardioembolic Stroke

Kadian M[1,2], Kok C[1,3], Dupuy A[4,5], Liu X[4,5], Clayton Z[1,3], Ravindran D[1,3], Rao R[1,3], Curnow J[6], Chong J[1,3,7], Passam F[4,5], Pasalic L[6], Kizana E[1,3,7]
[1]The Centre for Heart Research, The Westmead Institute for Medical Research, Westmead, Australia, [2]The University of Sydney, Camperdown, Australia, [3]Westmead Clinical School, The University of Sydney, Westmead, Australia, [4]Central Clinical School, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia, [5]The Heart Research Institute, Charles Perkins Centre, Camperdown, Australia, [6]Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Sydney Centres for Thrombosis and Haemostasis, Westmead Hospital, Westmead, Australia, [7]Department of Cardiology, Westmead Hospital, Westmead, Australia

Aim: Cardiac thromboembolism persists as the culprit behind most ischemic strokes; a majority arising from the left atrial appendage. In order to combat this disease and overcome limitations of systemic oral anticoagulants, we aimed to develop a novel anticoagulant viral vector to focally target cardiac regions of increased thrombotic potential, thereby presenting an alternative for long-term anticoagulation.

Method: The anticoagulant phenotype of these vectors was functionally assessed through in vitro and ex vivo platforms. For in vitro testing, a novel, cell-based version of the calibrated automated thrombogram assay, coupled with the overall haemostatic potential assay were utilised to determine thrombokinetics in endothelial cell culture models. For ex vivo testing, freshly isolated and characterized porcine left atrial appendage endothelial cells were used. These cells were used to endothelialise microfluidic devices, which were stimulated with TNF-α prior to perfusion with human whole blood. Changes in fibrin, platelets and neutrophils were investigated in the presence and absence of our novel anticoagulant viral vector.

Results: Experimental findings from our *in vitro* work showed a significant* decrease in the endogenous thrombin potential of vector-transduced cells, coupled with a significant* decrease in the velocity of thrombogenesis. Furthermore, transduced cells had a marked* decrease in the peak rate of thrombin generation and a significant* increase in the time taken to achieve peak thrombin generation. Experimental findings from our *ex vivo* work highlighted significant* reductions in fibrin and platelets within the microfluidic model, as well as notable decreases in neutrophil populations.

*: $p \leq 0.0001$

Conclusion: These findings show the effects of our anticoagulant vectors in inhibiting thrombosis and help to guide the development of focal gene therapy strategies to target thrombosis at its point of origin in the prevention of cardioembolic-stroke

MTH1 protects platelet mitochondria from oxidative damage and is essential for GPCR-dependent platelet function and thrombosis

Qiao J[1], Ding Y[1], Gui X[1], Chu X[1], Zeng L[1], Andrews R[2], Gardiner E[2], Xu K[1]
[1]Xuzhou Medical University, Xuzhou, China, [2]Australian National University, Canberra, Australia

Aim: In this study, we aimed to investigate the role of MTH1 in platelet function and thrombosis.

Method: Megakaryocyte/platelet-specific MTH1 knockout mice were utilized to evaluate the role of MTH1 in platelet activation, aggregation, spreading, clot retraction, in vivo hemostasis, arterial and venous thrombus formation as well as mitochondrial ROS generation and DNA oxidative damage. Quantitative phosphoproteomic assay and non-targeted metabolomics assay were performed to investigate the molecular mechanism. The role of MTH1 in human platelet function was also assessed by using MTH1 inhibitor.

Results: We first detected MTH1 in both human and mouse platelets. MTH1 deficiency in platelets/megakaryocytes significantly prolonged tail-bleeding time and impaired both arterial and venous thrombus formation. Consistently, MTH1 deficiency significantly reduced platelet aggregation, phosphatidylserine exposure and calcium mobilization induced by thrombin but not by collagen-related peptide (CRP) even at a higher dose along with decreased mitochondrial ATP production. Thrombin but not CRP induced Ca2+-dependent mitochondria reactive oxygen species generation. Further, MTH1 deficiency significantly impaired platelet metabolism after thrombin stimulation as shown by a non-targeted metabolomics assay. Quantitative phosphoproteomic analysis revealed dysregulated phosphorylation of mitochondrial proteins involving in the regulation of platelet metabolism and mitochondrial protein synthesis. Mechanistically, MTH1 deficiency caused oxidative damage to platelet mitochondrial DNA and reduced the expression of cytochrome c oxidase 1 (MT-CO1) (a core subunit of complex IV in the mitochondrial respiration chain) in thrombin-stimulated platelets. Furthermore, inhibition of MTH1 decreased human platelet aggregation and increased mitochondrial DNA oxidative damage after thrombin stimulation.

Conclusion: Our study identifies a novel regulatory role for MTH1 in platelet function and thrombus formation. This is an important protective mechanism against oxidative stress in platelets and supports MTH1 as a new potential therapeutic target for prevention of thrombotic or cardiovascular diseases.

T-cell receptor sequencing confirms long-term in vivo persistence of adoptively transferred donor-derived pathogen and tumour antigen specific T-cells for myeloid malignancies in recipients of stem cell transplant (the INTACT trial)

Blyth E[1,2,6], Jiang W[2,6], Myers N[2], Gloss B[2], Bateman C[2,4,6], Avdic S[2,6], Clancy L[5], Street J[2], Simms R[2,6], Lee K[1,2,6], Gabriel M[5], Micklethwaite K[1,2,3,6], Gottlieb D[1,2,6]

[1]Blood transplant and Cell Therapy Program, Department of Haematology, Westmead Hospital, Westmead, Australia, [2]Westmead Institute of Medical Research, University of Sydney, Westmead, Australia, [3]Blood Transplant and Cell Therapy Laboratory, ICPMR, NSW Health, Westmead, Australia, [4]Department of Oncology, Children's Hospital and Westmead, Westmead, 2145, [5]Research and Education Network, Western Sydney Local Health District, Westmead, Australia, [6]Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Camperdown, Australia

Title: T-cell receptor sequencing confirms long-term *in vivo* persistence of adoptively transferred donor-derived pathogen and tumour antigen specific T-cells for myeloid malignancies in recipients of stem cell transplant (the INTACT trial)

Background: We performed a clinical trial of donor-derived adoptive T-cell therapy in patients undergoing allogeneic haemopoietic stem cell transplant (HSCT). The INTACT trial involved a novel combination of donor-derived, tumour-associated antigen specific T-cells targeting WT1 and PRAME, and multipathogen T-cells targeting CMV, EBV, Adenovirus and Aspergillus given prophylactically to reduce infection and relapse in patients undergoing HSCT for myeloid malignancies. Favourable clinical outcomes have been previously reported; 8/10 patients who received a total of 38 T-cell infusions are alive and in remission without clinically significant infections at a median of 740 days post-transplant.

Aim: To characterise the *in vivo* activity of adoptively transferred multipathogen and myeloid tumour antigen specific T-cells using T-cell receptor (TCR) sequencing.

Methods Multipathogen and myeloid tumour antigen specific T-cells were manufactured from fully matched related or unrelated transplant donors. To identify T-cell clones specific for each target, products were restimulated with antigen and sorted by FACS using activation markers CD137 or CD154. The complementarity determining region 3 of the TCR chain was amplified and sequenced from the extracted genomic DNA from sorted products, donor and recipient samples using a commercial kit.

Results: In all cases, clones for each specificity were detectable after infusion apart from one where no WT1-specific T-cell clones could be identified. Product clones were noted to expand immediately after infusion and persisted to the end of follow up. Antigen stimulated expansion of product-derived clones was noted in a case of CMV reactivation where MHC tetramer+ cells included product clones that had been identified by TCR sequencing. *In vivo* expansion after T-cell infusion was also observed in cases of EBV, *Aspergillus*, PRAME and WT1 specific clones.

Conclusion: Our findings strongly support that adoptively transferred *ex vivo* expanded T-products targeting both infectious and malignant antigens engraft in recipients and persist long-term.

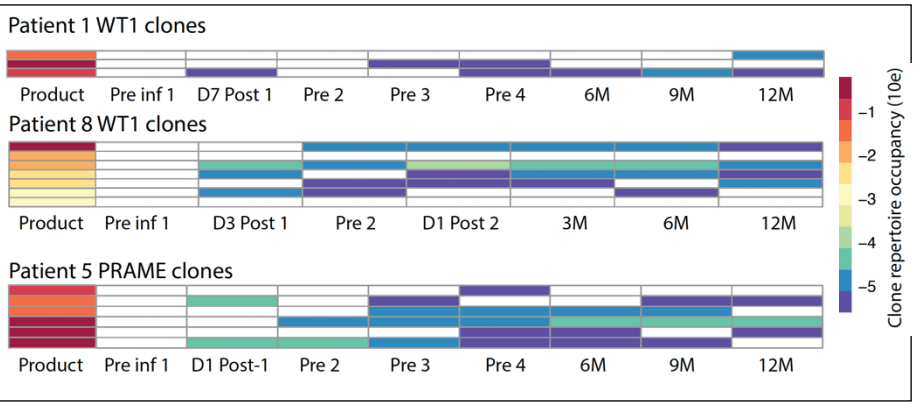


Figure 1. Clone tracking of tumour antigen specific T-cells. Each row is a unique clonotype and its presence and clonal proportion within each sample is shown. "Pre" or "Post-"= timepoints of up to 4 WT1/PRAME infusions; M = months

Genomic insights into primary refractory multiple myeloma: Exploring circulating cell-free tumour DNA analysis

Mithraprabhu S[1], Reynolds J[1], Quach H[2], Horvath N[3], Kerridge I[4], Lim S[1], Wong D[1], Khong T[1], Durie B[5], Spencer A[1]

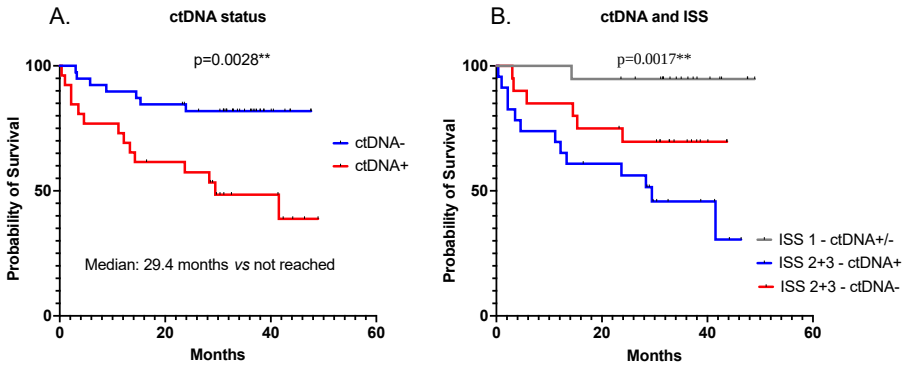
[1]Alfred Health - Monash University, Melbourne, Australia, [2]3St.Vincent's Hospital Melbourne, University of Melbourne, , Melbourne, Australia, [3]Royal Adelaide Hospital, Adelaide, Australia, [4]Royal North Shore Hospital, Sydney, Australia, [5]Cedars-Sinai Comprehensive Cancer Center, Los Angeles, United States of America

Aim: Approximately 20% of newly diagnosed multiple myeloma (MM) patients exhibit resistance (primary refractory) or sub-optimal response (SOR) based on International Myeloma Working Group (IMWG) response criteria to standard of care treatment. Our aim was to gain genomic insights utilising minimally invasive circulating cell-free tumour DNA analysis in these patients.

Method: We analysed 69 primary refractory or SOR patients enrolled in the ALLG MM17 (ACTRN12615000934549) and MM21 trials (ACTRN12618001490268). Peripheral blood was collected at study entry and relapse using Streck DNA BCT tubes for cell-free DNA extraction. Blood in EDTA tubes were collected at study entry for isolating mononuclear cells and extracting DNA as germline control. Custom-designed targeted amplicon sequencing of 22-genes was performed on 157 DNA samples. Bioinformatics analysis to determine somatic mutations involved CLC Genomics Workbench v21 and QCI Interpret. Mutational profiles were correlated with progression-free survival (PFS) and integrated with the International Staging System (ISS). We observed the kinetics of mutations at relapse compared to study entry.

Results: Preliminary analysis revealed a higher incidence of *KRAS/NRAS/BRAF* (RAS/RAF; p=0.04) and *ATM/ATR/TP53* (DNA damage repair/DDR, p=0.01) mutations in patients who relapsed compared to non-relapse patients. We categorised patients with RAS/RAF and/or DDR mutations as ctDNA+ or ctDNA- (absence of these mutations) and correlated this with PFS. The ctDNA+ patients exhibited significantly shorter PFS than ctDNA- patients (p<0.005, Figure A). Integration of ctDNA status with ISS staging revealed that ISS 2+3/ctDNA+ patients had significantly shorter PFS compared to ISS 2+3/ctDNA- and ISS 1/ctDNA+/- patients (p<0.005, Figure B). Longitudinal monitoring of ctDNA mutations showed that in 89% of patients, dominant mutations present at relapse were already detectable at the start of secondary therapy.

Conclusion: In conclusion, ctDNA analysis identifies potentially targetable mutations, predicts outcomes, improves risk-stratification and has the potential to guides therapy decisions, thereby advancing personalised medicine in MM.



Tracking in vitro development of RUNX1+ haemogenic endothelium

Saxena R[1,2,3], Labonne T[1,3], Ng E[1,2,3], Stanley E[1,2,3], Elefanty A[1,2,3]
[1]Murdoch Children's Research Institute, Parkville, Australia, [2]University of Melbourne, Parkville, Australia, [3]The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Parkville, Australia

Aim: Allogeneic haematopoietic stem cell (HSC) transplantation is a critical therapy in many blood disorders however, many patients are unable to find a perfectly matched donor. The in vitro generation of HSCs from patient derived induced pluripotent stem cells (iPSCs) would allow generation of perfectly matched HSCs for therapy.

Context: In the embryo, HSCs are initially generated from the aorta-gonad-mesonephros region, from a specialised haemogenic endothelium (HE) through an endothelial to haematopoietic transition (EHT). Our incomplete understanding of HSC specification from HE, coupled with the lack of specific markers for HE or HSCs has impacted our ability to characterise and control the EHT and hence, the development of HSCs in vitro.

Method: RUNX1 is an essential regulator of haematopoiesis and one of the first markers of haemogenic potential. We generated a RUNX1 iPSC reporter line, and differentiated the cells into haematopoietic lineages to generate RUNX1+ HE and HSC-like cells. Key cell surface markers were analysed using flow cytometry and RUNX1::mCHERRY expression localised with confocal imaging. The blood cells were then transplanted into immunodeficient mice for functional validation.

Results: RUNX1+ HE cells appeared from day 5 of differentiation, as a subset of arterial endothelium that downregulated CXCR4 expression. By day 10, endothelial cells acquired a haemogenic identity marked by RUNX1+ CD34+ CXCR4- CD73lo expression and appeared to accumulate on the outside of the swirling embryoid bodies. By day 14, abundant RUNX1+ blood cells were shed into the medium. The d14 blood cells were capable of multi-lineage reconstitution in mice.

Conclusion: The RUNX1 reporter line will enable us to further dissect the development of HE and present an opportunity to study the emergence of HSCs with long-term, multi-lineage engraftment.

Interim analysis of a nation-wide, investigator-initiated trial of glofitamab plus R-CHOP or polatuzumab vedotin-R-CHP in patients ≤65 years of age with high-risk DLBCL: COALITION

Minson A[1,2,3], Verner E[4], Giri P[5], Wong S[6], Ratnasingam S[7], Butler J[8], Janowski W[9], Ku M[10], Cheah C[11], Hertzberg M[12], Herbert K[13], Hamad N[14], Yannakou C[15], Neeson P[1], Saghebi J[1], Blombery P[1,2,3], Robertson M[1,3], Lau L[1], Xie J[1], Seymour J[1,2,3], Dickinson M[1,2,3]

[1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Royal Melbourne Hospital, Melbourne, Australia, [3]University of Melbourne, Melbourne, Australia, [4]Concord Repatriation Hospital, Concord, Australia, [5]Royal Adelaide Hospital, Adelaide, Australia, [6]The Alfred Hospital, Melbourne, Australia, [7]University Hospital Geelong, Geelong, Australia, [8]Royal Brisbane and Women's Hospital, Herston, Australia, [9]Calvary Mater Newcastle, Waratah, Australia, [10]St Vincent's Hospital, Fitzroy, Australia, [11]Sir Charles Gairdner Hospital, Nedlands, Australia, [12]Prince of Wales Hospital, Randwick, Australia, [13]Cabrini Hospital, Malvern, Australia, [14]St Vincent's Hospital, Darlinghurst, Australia, [15]Epworth HealthCare, Melbourne, Australia

Aim: Improved treatments are needed for patients with high-risk (HR) DLBCL, in whom durable responses to R-CHOP are <50%. We present an interim analysis of an ongoing investigator-initiated, parallel-arm, phase I/II study of the CD20/CD3 bispecific antibody glofitamab in combination with R-CHOP or Polatuzumab vedotin (Pola)-R-CHP in younger patients with HR-DLBCL.

Method: Eligibility included newly-diagnosed DLBCL, age 18-65 years, and at least one HR feature: IPI ≥3, NCCN-IPI ≥4, or *MYC*/*BCL2* and/or *BCL6* rearrangement. Enrolment after 1 cycle of R-CHOP and ECOG <4 at baseline was permitted. Patients received 5 cycles of glofitamab with either R-CHOP (Arm A) or Pola-R-CHP (Arm B), followed by 2 cycles of glofitamab consolidation.

Primary endpoints are safety, relative dose intensity (RDI) and rate of treatment discontinuation. Responses are evaluated after cycles 2, 4 and 6 and then 3-6 monthly.

Results: 73/80 patients have been recruited at 14 Australian sites; 46 are included for interim evaluation. Median age was 52 (range 24-65) years. Median total metabolic tumour volume was 673cm^3 and median time to treatment was 15 days. Grade ≥3 adverse events were observed in 59% (Table 1). Cytokine release syndrome (CRS) was observed in 24% and was grade 1 in all but one patient. There were 3 dose interruptions to glofitamab but no glofitamab or early chemotherapy discontinuations. The RDI was above 90% for all major treatment components. ORR after cycle 6 was 96% (74% CR, 22% PR). With median follow-up 8.1 months the estimated OS and PFS at 12 months was 89%/91% for Arm A and 100%/76% for Arm B (Figures 1&2).

Conclusion: Glofitamab with R-CHOP or Pola-R-CHP was deliverable with low-grade CRS and maintenance of RDI. The ability to enrol after 1 cycle of R-CHOP resulted in short time to treatment initiation and inclusion of patients with high-burden disease. Efficacy appears promising in this HR population.

Table 1: Safety profile

Safety overview	Arm A (25)	Arm B (21)	Total (46)
Adverse events (any grade)	25/25 (100%)	21/21 (100%)	46/46 (100%)
Serious adverse events	10/25 (40%)	12/21 (57%)	22/46 (48%)
Related to glofitamab	7/25 (28%)	8/21 (38%)	15/46 (33%)
Related to polatuzumab	N/A	8/21 (38%)	8/46 (17%)
Grade >=3 adverse events	13/25 (52%)	14/21 (67%)	27/46 (59%)
Febrile neutropenia	2/25 (8%)	7/21 (33%)	9/46 (20%)
Infections	13/25 (52%)	16/21 (76%)	29/46 (63%)
Grade>=3	2/25 (8%)	5/21 (24%)	7/46 (15%)
Cytokine release syndrome			
Grade 1	5/25 (20%)	5/21 (24%)	10/46 (22%)
Grade 2	1/25 (4%)	0/21 (0%)	1/46 (2%)
Peripheral neuropathy			
Grade 1	12/25 (48%)	9/21 (43%)	21/46 (46%)
Grade 2	2/25 (8%)	1/21 (5%)	3/46 (7%)
Neurotoxicity/ICANS	0 (0%)	0 (0%)	0 (0%)

Figure 1: PFS

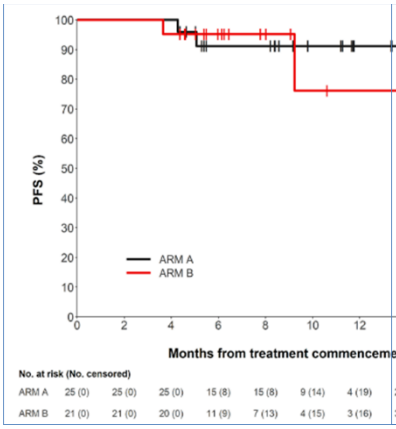
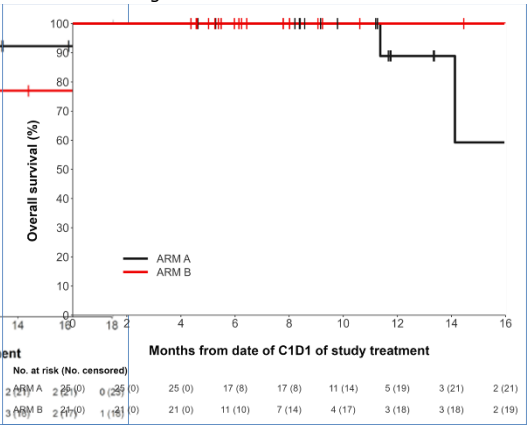


Figure 2: OS



Harnessing the stress response for immunity in acute myeloid leukaemia

Salik B[1,2], Straube J[1], Lee C[1], Lane S[1,2], Nakamura K[1,3]
[1]Cancer Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]School of Medicine, University of Queensland, Brisbane, Australia, [3]School of Biomedical Sciences, University of Queensland, Brisbane, Australia

Aim: Acute myeloid leukaemia (AML) is an aggressive cancer of the blood with dismal survival outcomes. The curative potential of the allogeneic hematopoietic stem cell transplant highlights the importance of immune-mediated control in AML. However, the importance of natural immune surveillance against AML remains to be fully characterized. We hypothesized that regulation of the integrated stress response (ISR), a critical adaptive mechanism, is an important determinant of immune response to AML.

Methods and Results: Using a murine model of a poor prognosis AML subset driven by the t(9;11)(p22;q23) translocation (MLL-AF9 hereafter referred to as MA9), we have identified that regulation of the ISR by GADD34 protects AML from anti-tumour immunity. While both control and GADD34$^{-/-}$ MA9 AML progressively grew in immunodeficient hosts (*Rag2*$^{-/-}$*Il2r*$^{-/-}$ mice), GADD34$^{-/-}$ MA9 AML but not control MA9, was effectively controlled in immunocompetent wild type or natural killer (NK) cell-competent hosts (*Rag2*$^{-/-}$ mice) (Log-rank Mantel-Cox test). Similarly, antibody-mediated depletion of NK and CD8^{+} T cells exacerbated GADD34$^{-/-}$ MA9 AML development but mice with intact immunity remained tumour free. RNA-sequencing and proteomic analyses on enriched leukemic cells demonstrated that GADD34$^{-/-}$ AML is metabolically distinct. Our ex-vivo data suggest that GADD34-mediated regulation of the ISR contributes to cellular redox control where both GADD34-deficient murine and human AML model systems are marked by elevated intracellular reactive oxygen species (ROS). Indeed, ROS accumulation potentiates the apoptotic cascade and immune-derived cytokines are potent inducers of ROS. In this light, GADD34 deficient murine and human AML cell lines were susceptible to immune-mediated toxicity either by T-cell conditioned medium or by co-culture with NK cells. Importantly, clinical data support that ISR maybe tightly associated with immunity in AML.

Conclusion: Our findings establish a novel link between tumour ISR and anti-tumour immunity indicating that therapeutic interventions targeting this pathway could potentially increase the sensitivity of AML to cancer immunotherapy.

Blinatumomab in combination with an intensive paediatric protocol results in high rates of end-consolidation MRD negativity – update of the Australasian Leukaemia and Lymphoma Group (ALLG) ALL09 "SUBLIME" Study

Greenwood M[1], Gangatharan S[2], Osborn M[3], Ng A[4], Fleming S[5], Fedele P[6], Trahair T[7], Casey J[8], Mapp S[9], Cheung C[10], Armytage T[11], Henderson M[12], Sutton R[12], McCloud P[13], Larsen S[14], Presgrave P[15], Kwan J[16], Bennett S[17], Fong C[18], Dalla Pozza L[19]

[1]Royal North Shore Hospital and The University of Sydney, Sydney, Australia, [2]Fiona Stanley Hospital, Perth, Australia, [3]Department of Haematology & Oncology, Women's & Children's Hospital, Adelaide, Australia, [4]Department of Clinical Haematology, Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Melbourne, Australia, [5]The Alfred Hospital Melbourne and Monash University, Melbourne, Australia, [6]Monash Hospital, Melbourne, Australia, [7]Sydney Children's Hospital, Randwick, Australia, [8]The Townsville Hospital, Townsville, Australia, [9]Princess Alexandra Hospital, Brisbane, Australia, [10]Prince of Wales Hospital, Sydney, Australia, [11]Gosford Hospital, Gosford, Australia, [12]Children's Cancer Institute, University of NSW, Randwick, Australia, [13]McCloud Consulting Group, Sydney, Australia, [14]Institute of Haematology, Royal Prince Alfred Hospital, Sydney, Australia, [15]Wollongong Hospital, Wollongong, Australia, [16]Blood Transplant and Cellular Therapies Program, Westmead Hospital, Sydney, Australia, [17]The Canberra Hospital, Canberra, Australia, [18]Department of Haematology, Austin Hospital, Heidelberg, Australia, [19]The Children's Hospital at Westmead, Sydney, Australia

Aim: To present updated results of the ALL09 study which assessed the impact of blinatumomab on day 79 (TP2) MRD^{neg} rates when used in protocol I phase 2 (consolidation) of a BFM-based protocol in *de novo* AYA CD19+ ALL.

Method: Patients aged between 15 and 39 years with *de novo* CD19+ Ph-negative ALL were eligible. ALL09 replaced cyclophosphamide, cytarabine and 6MP in protocol I and II phase 2 of ALL06 with blinatumomab in a Simon 2-stage design using estimated TP2 MRD^{neg} rates from ALL06 as the comparator. Centralised MRD testing using RQ-PCR with sensitivity $\leq 10^{-4}$ at day 33 (TP1) and day 79 (TP2) of protocol I was reported using EuroMRD criteria. The primary endpoint (TP2 MRD^{neg} rate) was assessed using a modified intention to treat (mITT) population consisting of all patients with informative MRD commencing blinatumomab at day 36.

Results: 55 patients enrolled from 04/19-04/22 comprised the ITT cohort (Table 1). CR rate was 68% at day 15, 94.9% TP1 and 100% of evaluable pts at TP2. 18 patients proceeded to HR blocks, with 10 receiving allo-SCT. Of the ITT cohort, 4 had no MRD marker, 1 induction death (ID), 1 progressive disease, and 1 withdrew, leaving n=48 in the mITT cohort. TP1 MRD^{neg} was 34.0% and 70.8% at TP2, meeting the primary objective (p=0.037). In the ALL06 B-cell cohort, TP1 MRD^{neg} was 19.1% and 56% at TP2. As of last follow up, the ITT cohort had n=4 relapses (n=3 post-SCT) and 4 deaths (n=3 relapses with 2 post-SCT, n=1 ID), with n=2 relapses (n=2 deaths) in the mITT cohort.

Table 1: Clinical characteristics of the Intention to Treat cohort

Number of Patients	Median Age in years (range)	Male	Female	Median days follow up (range)	t(4;11)
55	25 (16-39)	30	25	522 (274-806)	4

Conclusion: Blinatumomab was efficacious in improving TP2 MRD^{neg} rates when compared to the ALL06 study using a Simon's 2-stage design. These findings will be taken forward in future ALLG studies in AYA ALL.

High expression of DUX4 mRNA is a reliable indicator of DUX4-rearranged acute lymphoblastic leukaemia

Rehn J[1,2], Schutz C[1], Page E[1,2], Heatley S[1,2], Eadie L[1,2], McClure B[1,2], Breen J[2,3], Yeung D[1,2,4,5], White D[1,2,5]

[1]Precision Cancer Medicine, SAHMRI, Adelaide, Australia, [2]Faculty of Health and Medical Science, University of Adelaide, Adelaide, Australia, [3]Telethon Kids Institute, Adelaide, Australia, [4]Haematology Department, Royal Adelaide Hospital, Adelaide, Australia, [5]Australasian Leukaemia & Lymphoma Group (ALLG), Richmond, Australia

Aim: *DUX4* rearrangements (*DUX4*r) comprise a genomic subtype of B-cell acute lymphoblastic leukaemia (B-ALL) currently detectable only via next-generation sequencing. We aimed to characterise the genomic landscape of Australian *DUX4*r patients and develop a semi-quantitative reverse transcriptase PCR assay (RT-qPCR) to detect *DUX4* mRNA expression.

Method: mRNA-sequencing (mRNA-seq) was performed on B-ALL samples (n=725), and subtypes were assigned according to identified fusion genes, single nucleotide variants and gene expression profiles. Gene deletions were detected by multiplex ligation-dependent probe amplification (SALSA MLPA P202 and P335). SYBR Green RT-qPCR was used to determine *DUX4* mRNA expression. Wilcoxon rank sum test (*DUX4* expression) and Fisher's exact test (mutation burden) were used for assessing significance between *DUX4*r and non-*DUX4*r samples.

Results: Gene expression profiling identified *DUX4*r in 7.1% of B-ALL patients (comprising children 7% (n=24/341), adolescent young adults 12.3% (n=25/203), adults 1.7% (n=3/181)). *DUX4*r primarily involved *IGH* (n=24) but also rearrangement with *ERG* (n=1), *FRG2B* (n=3) and *BRPF1* (n=1). MLPA (n=46/52) revealed that *IKZF1* (26%) and *PAX5* deletions (19.6%) were less frequent than *CDKN2A/B* (45.6%). *ERG deletions* were highly enriched but not exclusive to *DUX4*r cases (31.1% vs 0.6%, p<0.001), as were Ras mutations (*NRAS, KRAS, PTPN11*; 46.2% vs 23.5%, p<001). Accurate quantification of *DUX4* expression is complicated by the presence of multiple pseudo-copies of *DUX4* in the reference genome. To overcome this issue, we utilised a custom transcriptome revealing that *DUX4* expression by mRNA-seq was significantly higher in patients with *DUX4*r (p<0.0001). A relative RT-qPCR assay confirmed higher expression of *DUX4* (median relative fold-change 3448 vs 104, p<0.001) in *DUX4*r (n=51) cases compared with samples of other B-ALL genomic subtypes (n=20).

Conclusion: We have characterised the genomic landscape of a large cohort of *DUX4*r samples. High expression of *DUX4* rather than deletion of *ERG* was identified as a defining feature of *DUX4*r patients.

STING agonists exert a direct anti-leukaemic effect in AML via induction of apoptosis

Yuan Y[1,2,3], Diepstraten S[1,2], Brown F[1,2], Morrow B[5], Matthews G[5], Devlin M[5], Strasser A[1,2], Roberts A[1,2,3,4], Kelly G[1,2], Wei A[1,2,3,4]

[1]Blood Cells and Blood Cancer Division, WEHI, Melbourne, Australia, [2]Department of Medical Biology, University of Melbourne, Melbourne, Australia, [3]Department of Clinical Haematology, Peter MacCallum Cancer Centre, Melbourne, Australia, [4]Department of Clinical Haematology, The Royal Melbourne Hospital, Melbourne, Australia, [5]Aculeus Therapeutics, Melbourne, Australia

Aim: Acute myeloid leukaemia (AML) remains a blood cancer with poor prognosis for which new therapeutic options are required. Agonists of STING (stimulator of interferon genes) are a new class of anti-cancer drugs under investigation.[1,2] Whilst the predominant interest in STING agonists have been their potential to augment anti-tumour immune responses, recent evidence suggests that these agents can also induce apoptosis.[3] We aimed to investigate whether STING agonists could have a cell-intrinsic effect in AML.

Method: We tested two small molecule STING agonists, ACU2086 (Aculeus, Melbourne) and GSK3745417 (purchased from SYNthesis Med Chem). Drug sensitivity assays were performed in six different human AML derived cell lines as well as primary AML specimens (fresh or cryopreserved bone marrow mononuclear cells). Cells were cultured for 48 hours and cell death assessed by PI/DAPI staining. Sensitivity assays were also performed in *Bax/Bak* double knock-out (KO) and *STING*-KO cells to confirm the mechanism of cell death.

Results: ACU2086 and GSK3745417 were active in 5/6 AML cell lines tested, including venetoclax-resistant lines. Marked activity was also seen in 4/7 primary AML specimens (Figure 1). *Bax/Bak*-KO and *STING*-KO cell lines were resistant to both compounds, confirming that cell death occurred by apoptosis and was dependent on BAX/BAK as well as STING. *P53*-KO cells demonstrated the same sensitivity as *P53*-wildtype cells to both agonists.

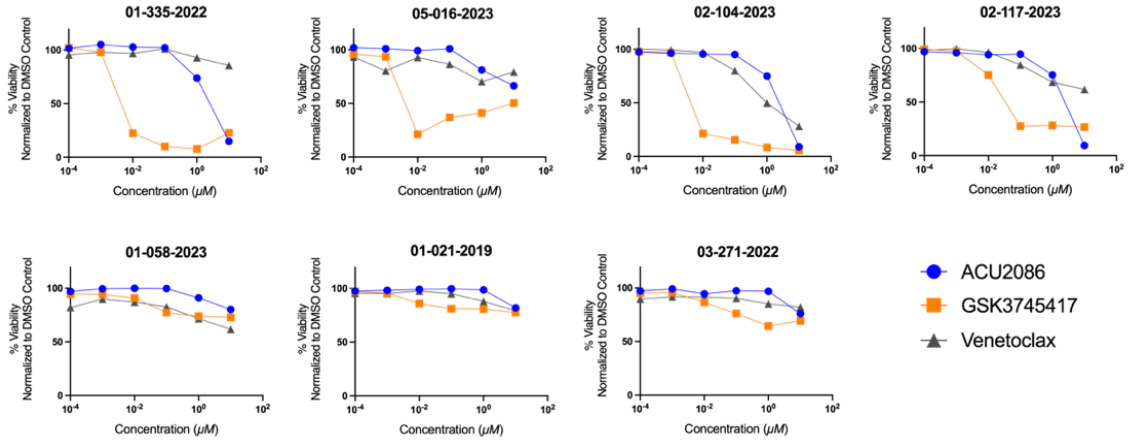


Fig 1. Activity of ACU2086, GSK3745417 and venetoclax in primary AML specimens (n=1).

Conclusion: STING agonists have a tumour-intrinsic cytotoxic effect in AML via induction of apoptosis and they demonstrate significant single agent activity in human AML cell lines and primary specimens. Experiments using patient-derived xenograft models are underway to extend these findings to *in vivo* studies.

References

1. Kwon J, Bakhoum SF. The Cytosolic DNA-Sensing cGAS-STING Pathway in Cancer. *Cancer Discov*. 2020;10(1):26-39.
2. Le Naour J, Zitvogel L, Galluzzi L, Vacchelli E, Kroemer G. Trial watch: STING agonists in cancer therapy. *Oncoimmunology*. 2020;9(1):1777624.
3. Gulen MF, Koch U, Haag SM, et al. Signalling strength determines proapoptotic functions of STING. *Nat Commun*. 2017;8(1):427.

Blinatumomab with Reduced Intensity Chemotherapy for Older Adults with Newly Diagnosed Ph negative B-Precursor Acute Lymphoblastic Leukemia – Final Results of the ALLG ALL08 Study

Fleming S[1,2], Reynolds J[2], Bajel A[3], Venn N[4], Kwan J[5], Moore J[6], Yeung D[7], Pati N[8], Leahy M[9], Kollopara S[10], Berkahn L[11], Sutton R[4], Wei A[3,12], Greenwood M[13]

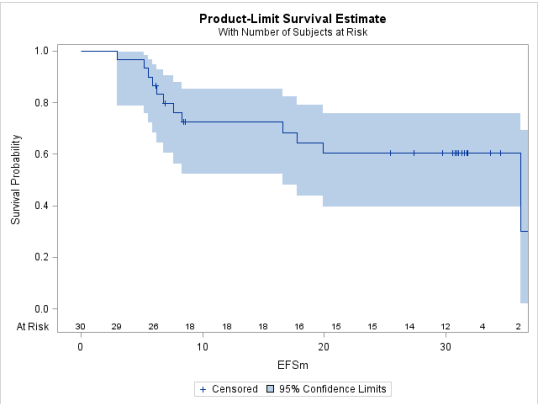
[1]Alfred Health, Melbourne, Australia, [2]Monash University, Melbourne, Australia, [3]Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Parkville, Australia, [4]Children's Cancer Institute, Randwick, Australia, [5]Westmead Hospital, Westmead, Australia, [6]St Vincent's Hospital Sydney, Darlinghurst, Australia, [7]Royal Adelaide Hospital, Adelaide, Australia, [8]Canberra Hospital, Canberra, Australia, [9]Royal Perth Hospital, Perth, Australia, [10]Australasian Leukaemia and Lymphoma Group, Richmond, Australia, [11]Auckland City Hospital, Auckland, New Zealand, [12]Walter and Eliza Hall Institute, Parkville, Australia, [13]Royal North Shore Hospital, St Leonards, Australia

Aim: Paediatric inspired regimens while highly effective in younger adults with Acute Lymphoblastic Leukaemia (ALL) are limited in application to older adults due to toxicity. The Australasian Leukaemia and Lymphoma Group (ALLG) ALL08 study (ACTRN12617000084381) was a phase 2 proof-of-concept study exploring Blinatumomab with reduced intensity (RI) chemotherapy for older adults with newly diagnosed B-cell precursor (BCP) ALL.

Method: Adults aged 40-65 years were enrolled. A steroid pre-phase and subsequent debulking chemotherapy was followed by 4 alternating cycles of Blinatumomab and B-cycles of Hyper-CVAD. Patients with high risk disease were recommended to receive allogeneic stem cell transplant, others received POMP maintenance therapy. Measurable residual disease (MRD) was assessed by PCR with sensitivity of at least $1x10^{-4}$. Proof-of-concept criterion was an event-free-status at 2-years of 63%.

Results: 30 subjects (21 (70%) Male) with a median age of 51.7 years (39.5 – 66.5 years) with 28 (87%) ECOG 0-1 time of study entry were enrolled. 97% of subjects attained composite CR (CR/CRi) with 28 (93%) attaining this by the end of 1B, and an additional subject by the end of cycle 2A. MRD response ($\leq 10^{-4}$) was achieved in 70% (19/27) subjects at the end of 1B and 83% (20/24) subjects at the end of 2B. The estimated EFS at 24 months was 60.4% (median 36.1 months) with OS at 24 months of 78.6% (median NR). This was insufficient to meet the pre-specified proof-of-concept criteria. The regimen was well tolerated with the major toxicity being infective (53 episodes of infection) along with 2 episodes of cytokine release syndrome and 7 of neurological toxicity.

Conclusion: Blinatumomab with chemotherapy demonstrated a high rate of morphological and MRD response. Despite failing to meet pre-specified proof of concept criterion, response and survival outcomes were encouraging, demonstrating the feasibility of the approach in BCP-ALL.



Novel clinicogenomic findings in the emerging entity of acute myeloid leukaemia with UBTF tandem duplications

Harrop S[1], Nguyen P[1], Wilson C[1], Ryland G[1], Tiong I[1], Blombery P[1]
[1]***Peter Maccallum Cancer Centre, Melbourne, Australia***

Aim: *UBTF* tandem duplications (*UBTF*-TDs) have recently been recognised as a recurrent mutation in AML. They are more common in paediatric AML (9%) compared to adult AML (1%) and are associated with mutations in *WT1* and *FLT3*. Most patients have morphological dysplasia present in one or more lineages and there is an associated poor prognosis with high rates of early relapse. We aimed to characterise the clinicogenomic features of patients with *UBTF*-TD AML to further understand this emerging entity and identify any novel features.

Method: 159 patients with newly diagnosed AML or MDS with excess blasts and known *WT1* and/or *FLT3* mutations were identified and underwent assessment with a custom designed assay involving PCR of *UBTF* exon 13 with fluorescent labelled 6-FAM primers followed by fragment analysis via capillary electrophoresis. Genomic features were also characterised using error-corrected targeted next generation sequencing including *UBTF* exon 13.

Results: 20 patients with *UBTF*-TDs were identified. Adult AML with *UBTF*-TDs had karyotypes and molecular profiles consistent with their paediatric counterparts. Normal karyotype (9/20) and trisomy 8 (3/20) were recurrent as reported previously, while other comutations seen included *TP53*, *JAK3* and *GATA2*. AML with myelodysplasia associated changes and high-grade myelodysplastic syndromes (MDS) were reported in 40% of patients (8/20). Immunophenotyping (where available) demonstrated frequent loss of CD34 in the blast population (7/8). We identified two patients who had persistent *UBTF*-TD detected despite being in morphological remission with no disease identifiable by multiparametric flow cytometry consistent with its presence in a preleukaemic subclone. One patient had comutation of *IDH2* and *UBTF* in a preleukaemic clone that responded to IDH2 inhibition.

Conclusion: We have described the clinicogenomic features of a large cohort of patients with the new entity of AML with *UBTF*-TD and expanded the phenotype of this emerging entity.

Liquid biopsy monitoring is more sensitive than alternative techniques in extramedullary multiple myeloma

Bingham N[1,2], Khong T[1,2], Mithraprabhu D[1,2], Spencer A[1,2]
[1]Alfred Health, Melbourne, Australia, [2]Monash University, Melbourne, Australia

Aim: Extramedullary disease (EMD) affects 30% of multiple myeloma (MM) patients, and predicts poor outcomes. Current detection utilises PET/CT; bone marrow (BM) biopsies and consensus response criteria (CRC) are limited as EMD is frequently non-secretory with minimal BM involvement. EMD has driver mutations (DM) in MAPK pathway (*KRAS*, *NRAS*, *BRAF*). These DM are detectable in cell free DNA (cfDNA); we now seek to characterise the role of cfDNA in EMD.

Method: Plasma collected in Streck DNA tubes. cfDNA extracted using QIAGEN QIAamp® Circulating Nucleic Acid Kit. DM identified by whole genome sequencing. Then droplet digital PCR was used on cfDNA at additional timepoints: prior to EMD development, after treatment and at relapse.

Results: 13 patients included. 100% had DM in cfDNA at EMD diagnosis. Variant allele frequency (VAF) range: 0.05-37.63%.

Eight had ≥2 cfDNA timepoints. Change in cfDNA VAF mirrored PET-CT response to therapy and relapse. cfDNA VAF after therapy was compared with PET/CT, CRC and EuroFlow minimal residual disease (MRD). cfDNA was complementary to PET/CT: 3 cases were incongruous: 2 had cfDNA+ve with PET/CT-ve, and 1 cfDNA-ve but PET/CT+ve. In secretory disease, cfDNA was more sensitive than CRC (cfDNA+ve in n=3 in CR) and MRD (40% of MRD-ve timepoints were cfDNA+ve).

Change in VAF was prognostic. Patients achieving cfDNA negativity had the longest remissions (median 23.5 months) compared to cfDNA positivity (median 6 months). In EMD relapse from cfDNA negativity, cfDNA became positive prior to overt relapse by PET/CT, CRC, or BM MRD. Finally, DM were detectable in cfDNA prior to EMD diagnosis (>2 years in one case). Liquid biopsies from an additional 24 patients are being analysed.

Conclusion: In EMD patients, the cfDNA VAF is complementary to PET/CT, more sensitive than BM MRD and CRC, and predicts relapse. DM exist in cfDNA prior to EMD; this may allow tailored monitoring and early intervention.

Predicting treatment outcome in multiple myeloma: The correlation with circulating tumour DNA-based molecular response and minimal residual disease

Mithraprabhu S[1], Reynolds J[1], Quach H[2], Horvath N[3], Kerridge I[4], Khong T[1], Durie B[5], Spencer A[1]
[1]Alfred Health - Monash University, Melbourne, Australia, [2]St.Vincent's Hospital, University of Melbourne, Melbourne, Australia, [3]Royal Adelaide Hospital, Adelaide, Australia, [4]Royal North Shore Hospital, Sydney, Australia, [5]Cedars-Sinai Comprehensive Cancer Center, Los Angeles, United States of America

Predicting treatment outcome in multiple myeloma: The correlation with circulating tumour DNA-based molecular response and minimal residual disease

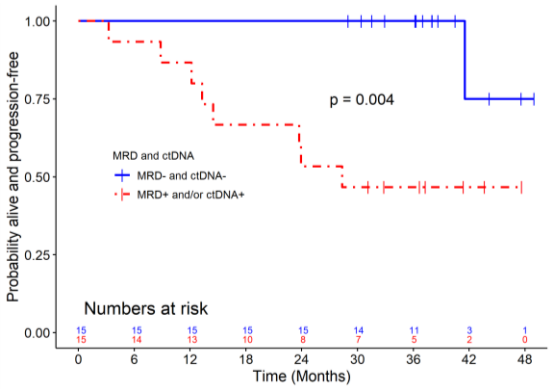
Aim:
We aimed to investigate the potential improvement in predicting treatment outcomes by incorporating the evaluation of circulating tumour DNA (ctDNA) into next-generation flow (NGF-EuroFlow™) minimal residual disease (MRD) analysis in multiple myeloma (MM).

Method: Peripheral blood plasma samples from 48 patients in the ALLG MM17 trial (ACTRN12615000934549) were analysed using high-sensitivity targeted amplicon sequencing of 22 MM-related genes. Samples were collected at baseline (B) and cycle 3 day 1 (C3D1) using Streck DNA tubes. The variant allele frequency (VAF) of mutations at B was used to calculate the fold change in VAF at C3D1, determining molecular response as ctDNA- (decrease in VAF) or ctDNA+ (increase in VAF). MRD assessment using EuroFlow™ was performed pre- and post-autologous stem cell transplant (ASCT) and at the end of the study. The ctDNA responses were combined with the best MRD and International Myeloma Working Group (IMWG) response criteria (< or □ complete response; CR) and correlated with progression-free survival (PFS) using Kaplan-Meier estimates.

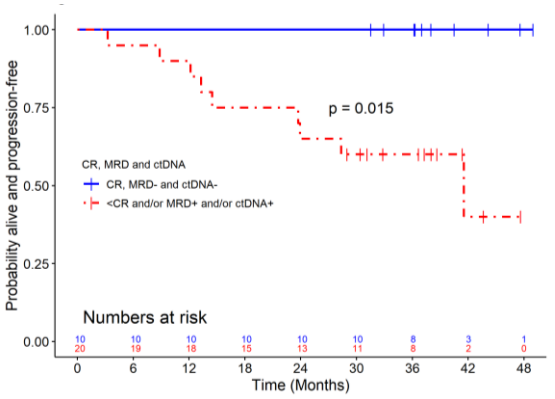
Results: Out of 48 patients, 41 had trackable mutations from B to C3D1. When patients with □CR and ctDNA- were compared with patients with <CR and/or ctDNA+, no significant PFS difference was seen (p=0.24). In contrast MRD-/ctDNA- patients demonstrated markedly superior PFS when compared to MRD+ and/or ctDNA+ patients (p=0.0040; Figure A). Finally combining all three response categories demonstrated that patients who were □CR, MRD- and ctDNA- also had a superior PFS compared to patients who were <CR and/or MRD+ and/or ctDNA+ (p=0.015; Figure B).

Conclusions: Our findings indicate that MM patients with a molecular response, characterised by a reduction in ctDNA mutational burden, and who are also MRD- have a better treatment outcome. This study confirms the utility of ctDNA-based early molecular response as a predictor of patient outcomes in MM.

A.



B.



Survival benefit of birtamimab in Mayo Stage IV AL amyloidosis in the Phase 3 VITAL clinical trial was consistent across all key baseline variables

Cohen A[1], Gertz M[2], Comenzo R[3], Kastritis E[4], Landau H[5], Libby E[6,7], Liedtke M[8], Sanchorawala V[9], Schönland S[10], Wechalekar A[11], Ando Y[12], Koh Y[13], Zonder J[14], Palladini G[15,16], Nie C[17], Karp C[17], Jin Y[17], Conrad A[17], Kinney G[17], Merlini G[15,16]

[1]Abramson Cancer Center, The Hospital of the University of Pennsylvania, Philadelphia, USA, [2]Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, USA, [3]Division of Hematology and Oncology, Tufts Medical Center, Boston, USA, [4]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [5]Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, USA, [6]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, USA, [7]Division of Medical Oncology, Department of Medicine, University of Washington, Seattle, USA, [8]Stanford Cancer Institute, Stanford, USA, [9]Amyloidosis Center, Boston University School of Medicine, Boston, USA, [10]Medical Department V, Amyloidosis Center, Universitätsklinikum Heidelberg, Heidelberg, Germany, [11]National Amyloidosis Centre, Division of Medicine, University College of London, Royal Free Hospital, London, United Kingdom, [12]Department of Amyloidosis Research Nagasaki International University, Sasebo, Japan, [13]Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea, [14]Barbara Ann Karmanos Cancer Institute, Detroit, USA, [15]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [16]Amyloidosis Research and Treatment Center, Fondazione IRCCS, Policlinico San Matteo, Pavia, Italy, [17]Prothena Biosciences Inc, South San Francisco, USA

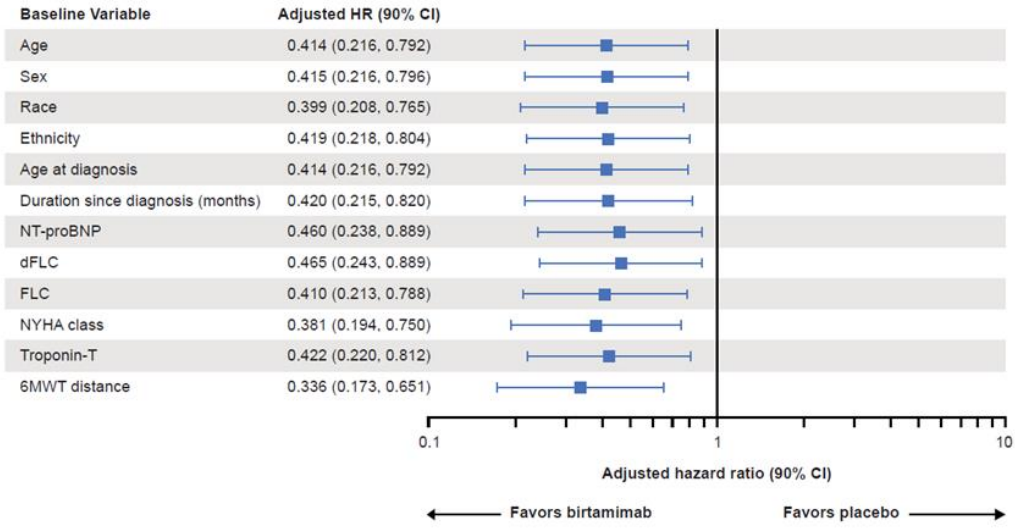
Aim: In AL amyloidosis, misfolded light chains (LCs) aggregate and deposit in vital organs. Patients with Mayo Stage IV disease have a median survival <6 months. Birtamimab binds □ and □ LCs, neutralizing circulating LCs and depleting deposited amyloid. The Phase 3 VITAL study (NCT02312206) of birtamimab in newly diagnosed, treatment-naïve AL amyloidosis patients with cardiac involvement was terminated per a futility analysis of the primary endpoint (time to all-cause mortality [ACM] or cardiac hospitalization). Post hoc analysis of ACM over 9 months revealed a significant survival benefit (HR=0.413 [95% CI: 0.191-0.895]) in Mayo Stage IV patients. We report ACM sensitivity analyses in Mayo Stage IV patients, adjusting for key baseline variables.

Method: ACM HRs and 90% CIs were estimated from the semi-parametric Cox regression model. Separately, key baseline variables were added to the model to evaluate impact on survival. Adjudicated deaths before 9 months were included; patients with no events were censored at 9 months.

Results: Among 260 patients, 77 (29.6%) had Mayo Stage IV disease (n=38 birtamimab + SOC; n=39 placebo + SOC). Baseline demographic and clinical characteristics were generally balanced between Mayo Stage IV treatment groups. After adjusting separately for each key baseline demographic, clinical, and laboratory variable, HRs ranged from 0.3360.465, with all upper bounds of 90% CIs <1 (**Figure**). Consistent with previous studies, birtamimab was generally well tolerated in Mayo Stage IV patients.

Conclusion: Birtamimab is the only investigational therapeutic to show a survival benefit in Mayo Stage IV AL amyloidosis. The survival benefit was consistent across all key baseline variables, reinforcing the strength of these data. The AFFIRM-AL study (NCT04973137), designed to confirm VITAL results in Mayo Stage IV AL amyloidosis, is being conducted under a Special Protocol Assessment agreement with the FDA with α=0.10 for the primary endpoint of ACM and is enrolling.

Figure. Forest plot of birtamimab survival benefit adjusted for key baseline variables for Mayo Stage IV patients – intent-to-treat population [9 months]



6MWT, 6-minute walk test; CI, confidence interval; dFLC, difference between involved minus uninvolved serum free light chains; FLC, free light chain; HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.

Optimisation of laser capture microdissection with tandem mass spectrometry for the diagnosis of amyloid subtype.

Rowland J[1,2], Loo-Oey D[3], Cuthbertson K[1], Lambie D[1,2], Gibbs S[4,5,6], Hoskings P[4], Kwok F[7,8], Shingde M[9], Mollee P[1,2]

[1]Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia, [2]Queensland Amyloidosis Centre, Princess Alexandra Hospital, Brisbane, Australia, [3]Translational Research Institute, Brisbane, Australia, [4]Eastern Health, Monash University Clinical School, Box Hill, Australia, [5]Epworth Freemasons Hospital, Melbourne, Australia, [6]Alfred Health, Melbourne, Australia, [7]Westmead Amyloidosis Service, Westmead Hospital, Sydney, Australia, [8]Department of Clinical Haematology, Westmead Hospital, Sydney, Australia, [9]Department of Tissue Pathology, Westmead Hospital, Sydney, Australia

Aim: Correct diagnosis of amyloidosis subtype is a critical step to direct patient management, inform prognosis and guide targeted genetic testing. Laser capture microdissection and tandem mass spectrometry (LMD-MS) analysis of formalin-fixed paraffin-embedded (FFPE) biopsy samples is emerging as the new gold-standard diagnostic technique in amyloid subtyping. A novel LMD-MS assay has been developed and implemented at Pathology Queensland, Australia, as a research tool. To allow transition to the clinical diagnostic laboratory, optimisation and validation of this assay was required.

Method: LMD-MS was performed on 78 patient samples and results evaluated assessing key MS measurements and the identification of amyloid signature and amyloid forming proteins. A bioinformatic reporting algorithm was developed and applied to a validation set of 226 samples. 121 samples were assessed by both IHC and LMD-MS for comparative assessment. 11 samples were assayed by this LMD-MS assay with results compared to those obtained at international laboratories.

Results: Critical MS datapoints contributing to confident amyloid forming protein identification were protein mean intensity, number of spectral matches and relative abundance. The addition of the Kabat protein library to the Swiss-Prot/Uniprot database did not interfere with identification of the amyloid proteins and showed superior diagnostic yield. Technical changes including buffer formulation and MS analyser technology variation had no impact on amyloid forming protein identification. A bioinformatic reporting algorithm was created which confidently identified an amyloid subtype in 96.1% of patient samples. LMD-MS was shown to be superior to IHC in subtyping of amyloid proteins with a non-diagnostic rate of only 3.6% The local LMD-MS assay showed 100% concordance with international laboratory analysis.

Conclusion: A novel LMD-MS assay has been developed and validated and shows superiority to the previous IHC based standard of care assessment and, while bioinformatic algorithm differs, gives identical information to LMD-MS from international centres. Role-out into the clinical diagnostic laboratory in Australia is planned.

An Australian Amyloidosis Network (AAN) analysis of Australian patients with AL amyloidosis treated with bortezomib-based chemotherapy

Gibbons A[1], Chakravorty L[3,4], Sidiqi H[3,4], Ting S[5], Ranjit Anderson N[1,2], Korczyk D[1,2], Mollee P[1,2], Gibbs S[5,6,7]

[1]Queensland Amyloidosis Centre, Princess Alexandra Hospital, Woolloongabba, Australia, [2]School of Medicine, University of Queensland, Brisbane, Australia, [3]Fiona Stanley Hospital, Perth, Australia, [4]Curtin Medical School, Curtin University , Perth, Australia, [5]Eastern Health Monash University Clinical School, Melbourne, Australia, [6]Epworth Freemasons, Melbourne, Australia, [7]Alfred Health, Melbourne, Australia

Aim: We aimed to describe the characteristics and outcomes of a uniformly treated cohort of Australian patients with AL amyloidosis.

Method: Cases were identified from the Fiona Stanley Amyloidosis Clinic, Queensland Amyloidosis Centre and Victorian and Tasmanian Amyloidosis Service. Subjects required a histological diagnosis of AL amyloidosis with confirmation of light chain subtype, symptomatic organ involvement and initial treatment with a bortezomib based regimen. Impact of baseline variables was assessed using Cox regression or long-rank testing as appropriate.

Results: 217 patients with AL amyloidosis were identified: Median age was 66 yrs and 45% were male. 76% of cases were lambda restricted, the median dFLC was 200mg/L, 76% had ≥ 10% bone marrow plasmacytosis and 8% had symptomatic myeloma. Cardiac stage was: 1 (14%), 2 (47%), 3A (26%) and 3B (13%). 68% had renal involvement with a median eGFR of 69mls/min/1.73m^2 and median proteinuria of 1.3g/d, with 7% dialysis dependent at presentation. Bortezomib regimens were: VCD (78%), VCD+daratumumab (10%), MDV (5%), VD (5%) and VRD (2%).

Best haematological response to bortezomib-based induction was: CR (31%), VGPR (34%), PR (15%) and less than PR (14%). 7% of patients died prior to response assessment. Median OS was 6.2 years. Worse OS was associated with more advanced cardiac disease (p=0.0001) but not higher dFLC (p=0.189) or size of the bone marrow plasmacytosis (p=0.825). Median OS by cardiac stage was stage 1 (not reached), stage 2 (95 months), stage 3A (73 months) and stage 3B (9 months).

Conclusion: Clinical characteristics of Australian patients with AL amyloidosis are similar to international series. Two thirds of patients achieve deep haematological responses with bortezomib-based therapy. We confirm the major impact of severity of cardiac involvement on survival in AL amyloidosis with stage 3B disease portending a particularly poor outcome.

The efficacy and safety of Prednisone, Cyclophosphamide, Doxorubicin and Carmustine(PCAB) in relapsed and refractory multiple myeloma(RRMM) in the era of novel therapy, including as a bridge to CAR-T therapy.

Li E[1], Bryant C[1], Jones E[1], King T[1], Talaulikar D[2], Ng J[2], Bryant A[3], Ridha Z[3], Wong Doo N[4], Ling S[3], Ho J[1], Joshua D[1]
[1]Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, Australia, [2]Department of Haematology, Canberra Health Services , Garran, Australia, [3]Department of Haematology, Liverpool Hospital, Liverpool, Australia, [4]Department of Haematology, Concord Repatriation General Hospital, Concord, Australia

Aim: Polychemotherapy has been replaced by novel therapies in the management of MM and is now reserved in the salvage setting despite limited published evidence. This study investigates the efficacy and safety of PCAB, a regimen established in the Australasian Leukaemia & Lymphoma Group Myeloma II trial[1], in RRMM.

Method: This multisite retrospective study involved four Australian tertiary hospitals. Patients with RRMM who received PCAB chemotherapy ± one novel agent between 1/1/2012-1/5/2023 were included. PCAB comprises of cyclophosphamide(600mg/m^2), doxorubicin(30mg/m^2) and carmustine(30mg/m^2) D1, and prednisone(60mg/m^2) D15, every 28 days. Baseline clinical characteristics, treatment response, adverse events and hospitalisation duration were recorded.

Results: Seventy-seven patients, median age 64 yrs(range 37-79) with median 4(range 1-8) prior lines of therapy were identified. Of these, 76.6% were exposed to both proteasome inhibitors and immunomodulatory drugs, 18.8% were exposed to daratumumab, and 63.6% had a previous autograft. Active extramedullary disease(EMD) and high-risk cytogenetics occurred in 35.1% and 58.0% of patients respectively. Forty-eight(62.3%) patients received only PCAB, while 29(37.7%) received it in combination, most commonly with bortezomib(n=14) or thalidomide(n=7). After median of 3 cycles(range 1-11), ORR was 38.4%(CR 5.5%) for the whole cohort, and 27.3% for those receiving PCAB alone. ORR in patients with EMD and high-risk cytogenetics were 30.8% and 42.9% respectively. Subsequent therapy was received in 72.7% of patients, including as bridge to CAR-T therapy(n=2), autograft(n=3) and allograft(n=3). Median PFS and OS was 4.0 and 6.7 months respectively.

Rate of grade 3/4 cytopenia and febrile neutropenia was 76.2% and 44.3% respectively. Treatment-related deaths, all due to infections occurred in six patients(7.8%). Patients were inpatient for median 3.6 days/cycle.

Conclusion: PCAB is effective in RRMM including in patients with high-risk cytogenetics and EMD. It may have a role as bridging therapy deliverable in an outpatient setting. Infection related mortalities highlight a need for careful monitoring in this heavily pre-treated cohort.

- Joshua DE, Penny R, Matthews JP, et al. Australian Leukaemia Study Group myeloma II: a randomized trial of intensive combination chemotherapy with or without interferon in patients with myeloma. *Br J Haematol* 1997;97(1):38-45.

Genomic Evolution and Resistance to Pirtobrutinib in Covalent BTK-Inhibitor Pre-treated Chronic Lymphocytic Leukemia Patients: Results from the Phase I/II BRUIN Study

Tam C[1], Brown J[2], Desikan S[3], Nguyen B[4], Won H[4], Tantawy S[3], McNeely S[5], Marella N[4], Ebata K[4], Woyach J[6], Patel K[7], Eyre T[8], Cheah C[9,10], Shah N[11], Ghia P[12], Jurczak W[13], Balbas M[4], Nair B[4], Abada P[4], Wang C[5], Wang D[4], Mato A[14], Gandhi V[3], Wierda W[3]

[1]Alfred Hospital, Melbourne, Australia, [2]Dana-Farber Cancer Institute and Harvard Medical School , Boston, USA, [3]University of Texas MD Anderson Cancer Center, Houston, USA, [4]Loxo@Lilly, , USA, [5]Eli Lilly and Company, , USA, [6]The Ohio State University Comprehensive Cancer Center, Columbus, USA, [7]Swedish Cancer Institute, Seattle, USA, [8]Churchill Cancer Center, Oxford University Hospitals NHS Foundation Trust, , UK, [9]Linear Clinical Research, Perth, Australia, [10]Sir Charles Gairdner Hospital, , Perth, Australia, [11]Medical College of Wisconsin, Milwaukee, USA, [12]Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, , IT, [13]Maria Sklodowska-Curie National Research Institute of Oncology, , PL, [14]Memorial Sloan Kettering Cancer Center, , USA

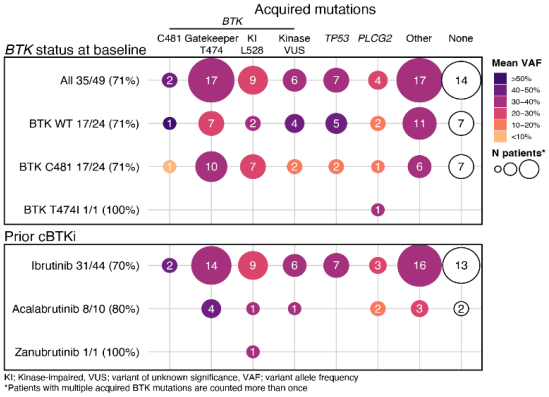
Aim: Pirtobrutinib, a non-covalent BTKi, demonstrated efficacy in patients with CLL resistant to cBTKi. Mechanisms of resistance to pirtobrutinib have not been systematically analyzed.

Method: Patients receiving pirtobrutinib monotherapy in phase1/2 BRUIN who developed disease progression (PD) were included. Targeted NGS of 74 genes was performed on PBMCs. Mutations were reported with a limit of detection (LoD) 5% variant allele frequency (VAF).

Results: 49 cBTKi pre-treated CLL patients who progressed on pirtobrutinib had paired NGS data at baseline and PD. Median number of prior lines: 4, 41 (84%) had discontinued prior cBTKi due to PD. Patients received ≥1 cBTKi: ibrutinib (n=44, 90%), acalabrutinib (n=10, 20%) or zanubrutinib (n=1, 2%). ORR to pirtobrutinib was 80%. Most common alterations at baseline were mutations in *BTK* (51%), *TP53* (49%), *ATM* (27%), *NOTCH1* (20%), *SF3B1* (18%), *PLCG2* (10%). *BTK* C481 VAF decrease/ clearance was observed at PD in most patients (92%, 22/24, median VAF decrease=100%). At PD, 71% (35/49) acquired ≥1 mutation, 55% (27/49) acquiring ≥1*BTK* mutation. Among these patients, 36 acquired *BTK* mutations were identified; including gatekeeper mutations (T474I/F/L/Y, 17/49, 35%), kinase-impaired (L528W, 9/49, 18%) and variants of unknown significance (VUS) (6/49, 12%; V416L (n=2), A428D (n=2), D539G/H (n=1), Y545N (n=1)) (Figure). Inspection for acquired *BTK* mutations at PD revealed 9 pre-existing mutations (8 patients) at low VAFs (1-4%): 6 gatekeeper T474I/L, 2 kinase-impaired L528W, 1 VUS A428D. These patients responded to pirtobrutinib (6/8, 75%ORR). Most-commonly acquired non-*BTK* mutations were *TP53* (7/49, 14%) and *PLCG2* (4/49, 8%).

Conclusion: Patients who progressed on pirtobrutinib showed clearance of *BTK* C481 clones and emergence/outgrowth of non-C481 clones. Many acquired *BTK* mutations pre-existed at baseline at low VAF, reflecting emergence on prior cBTKi and did not preclude pirtobrutinib efficacy. ~Half the patients did not acquire *BTK* mutations and 29% did not acquire any, suggesting alternate resistance mechanisms.

Figure. Number of patients with acquired mutations according to *BTK* status at baseline and prior type of cBTKi. The color gradient indicates the mean VAF at progression.



KI: Kinase-impaired, VUS: variant of unknown significance, VAF: variant allele frequency
*Patients with multiple acquired BTK mutations are counted more than once

Fixed-duration (FD) ibrutinib (Ibr) + venetoclax (Ven) for first-line treatment of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL): 4-year follow-up from the FD cohort of the phase 2 CAPTIVATE study

Tam C[1], Barr P[2], Allan J[3], Siddiqi T[4], Wierda W[5], Moreno C[6], Tedeschi A[7], Szafer-Glusman E[8], Zhou C[8], Abbazio C[8], Dean J[8], Szoke A[8], Ghia P[9]
[1]Alfred Health and Monash University, Melbourne, Australia, [2]Wilmot Cancer Institute, University of Rochester Medical Center, Rochester, USA, [3]Weill Cornell Medicine, New York, USA, [4]City of Hope National Medical Center, Duarte, USA, [5]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, USA, [6]Hospital de la Santa Creu i Sant Pau, Autonomous University of Barcelona, Barcelona, Spain, [7]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [8]AbbVie, North Chicago, USA, [9]Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, Milan, Italy

Aim: CAPTIVATE (NCT02910583) is a multicenter, phase 2 study of first-line Ibr+Ven in CLL/SLL. Follow-up from the FD cohort showed 3-year PFS rates of 88% overall and ≥80% in patients with high-risk features (Wierda, ASCO 2022). Updated results from the FD cohort with 4-year follow-up are presented.

Method: Patients aged ≤70 years with previously untreated CLL/SLL received 3 cycles of Ibr, then 12 cycles of Ibr+Ven (Ibr 420 mg/day orally; Ven ramp-up to 400 mg/day orally). Responses were investigator-assessed per iwCLL 2008 criteria. Undetectable minimal residual disease (uMRD; $<10^{-4}$) was assessed by flow cytometry.

Results: 159 patients were enrolled, including patients with high-risk features of unmutated IGHV (uIGHV) (56%), or del(17p) and/or *TP53* mutation (17%). Median time on study was 50 months (range 1–53). Four-year best CR rate was 58% and ORR was unchanged (96%); PFS and OS rates remained high, including in patients with high-risk features (**Table**). Four-year PFS rates by MRD status 3 months after stopping treatment were higher overall in patients with uMRD vs those with detectable MRD (dMRD) (**Table**). Median TTNT was not reached (range 1–53 months); the 4-year rate of freedom from next treatment was 84% (95% CI 77–89). After completing FD Ibr+Ven, 19 patients with PD initiated retreatment with Ibr; median time on retreatment was 11 mo (range 0–39). Of those, 1 had CR, 13 PR, and 1 each PR with lymphocytosis, SD, or PD. No new safety concerns were observed.

Conclusion: 4-year follow-up results of the CAPTIVATE study continue to support Ibr+Ven as an all-oral, once-daily, fixed-duration first-line regimen for CLL/SLL that provides deep, durable remissions with clinically meaningful PFS and time off treatment, including in patients with high-risk genetic features. Promising responses and consistent safety findings were observed upon retreatment with Ibr. EOT+3, 3 months after stopping treatment.

Table.	4-year PFS % (95% CI)	4-year OS % (95% CI)
FD Cohort (N=159)	79 (71–84)	98 (94–99)
del(17p) and/or *TP53* (n=27)	63 (41–79)	96 (76–99)
uIGHV (n=89)	73 (62–81)	97 (90–99)
uMRD at EOT+3, PB (n=90)	90 (81–95)	100
dMRD at EOT+3, PB (n=57)	66 (52–77)	100

Past informs the future: outcomes post allogeneic stem cell transplantation in chronic lymphocytic leukaemia (CLL)

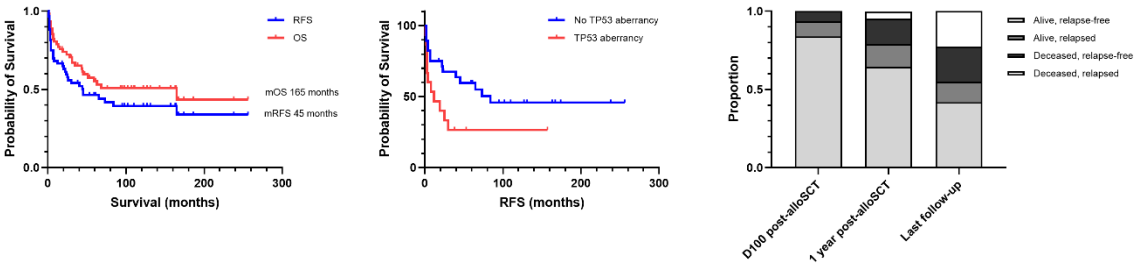
Bennett R[1], Frawley T[1], Khot A[1], Thompson P[1], Anderson M[1], Ritchie D[1]
[1]*Royal Melbourne Hospital & Peter MacCallum Cancer Centre, Melbourne, Australia*

Aim: Emerging immunotherapies for CLL, bispecific antibodies and CAR-T, may challenge the established role of allogeneic stem cell transplantation (alloSCT) for eligible patients. This retrospective study analysed characteristics and survival outcomes for patients with CLL following alloSCT, including impact of *TP53* aberrancy, over a 22 year period.

Method: All patients with CLL who underwent first alloSCT between 1/2000–4/2022 at Royal Melbourne Hospital (RMH) were identified from institutional alloSCT database. Additional patient details were extracted from institutional records. Relapse-free survival (RFS) and overall survival (OS) estimates/comparisons were analysed by Kaplan-Meier method/log-rank analyses on GraphPad Prism v.9.5.1.

Results: Sixty-two patients, median age 52 (range 25-75) years,79% male, were identified. Median two lines of therapy (range 1-9); 86% (n=49), 15.8% (n=9), and 19.3% (n=11) were purine-analogue-, venetoclax-, and BCR signalling inhibitor-exposed respectively. Seventeen (27.4%) had prior history of Richter transformation. Sixteen (35.6%) patients exhibited del(17p) +/- *TP53* mutation (5/10, all del[17p] positive). Sixty-four percent (n=38) were in PR (n=21) or CR (n=17) prior to alloSCT; 7/31 of whom had peripheral blood/bone marrow undetectable MRD by flow cytometry. Non-myeloablative/reduced-intensity conditioning was used for 71% (n=44) patients. Median RFS and OS were 45 (range, 0-256) months and 165 (range, 1-256) months respectively; 5-year RFS and OS rates were 46.5% and 55.5%. Presence of *TP53* aberrancy was associated with inferior mRFS,12m vs 84m (hazard ratio [HR] 2.3 [0.93-5.65], p=0.027). Failure to achieve CR/PR pre-alloSCT and previous RT were not associated with inferior RFS. One-year non-relapse mortality was 16.1% (n=10); 4/10 infection, and 3/10 GvHD. Incidence of grade 3/4 acute GvHD was 21.1% (n=12), and all chronic GvHD 65.5% (n=38).

Conclusion: RFS, OS, and NRM outcomes for CLL following alloSCT are consistent with international reports. Outcomes post-alloSCT for CLL exhibiting *TP53* aberrancy are suboptimal. These data serve as future comparator for local outcomes following newer immunotherapies.



Time-limited venetoclax-rituximab is effective for patients with BTKi-exposed CLL, but durable treatment-free remissions are uncommon

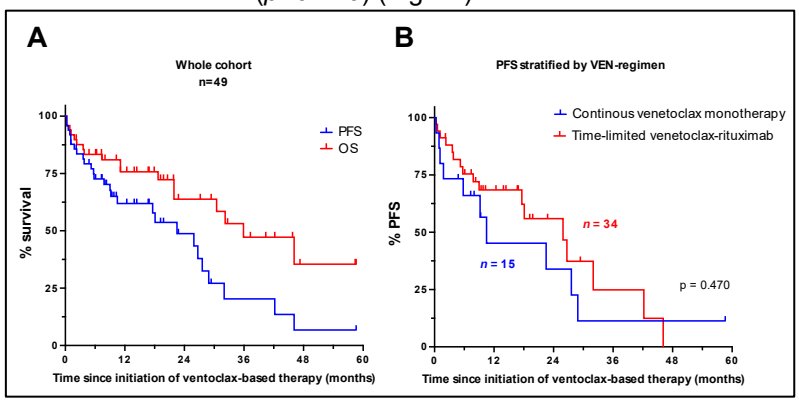
Lew T[1,2,3], Bennett R[1], Lin V[1,2,3], Whitechurch A[1], Hundunnetti S[4], Marlton P[4,5], Shen Y[6,7], Mulligan S[6,7,8], Casan J[1], Blombery P[1,3,9], Thompson E[9], Tam C[10], Roberts A[1,2,3], Seymour J[1,3], Thompson P[1], Anderson M[1,2]
[1]Peter MacCallum Cancer Centre And Royal Melbourne Hospital, Melbourne, Australia, [2]Blood Cells and Blood Cancer Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [3]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, Australia, [4]Department of Hematology, Princess Alexandra Hospital, Brisbane, Australia, [5]School of Medicine, University of Queensland, Brisbane, Australia, [6]Department of Haematology, Royal North Shore Hospital, Sydney, Australia, [7]Kolling Institute, Royal North Shore Hospital, Sydney, Australia, [8]Department of Haematology and Flow Cytometry, Laverty Pathology, Sydney, Australia, [9]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia, [10]Alfred Hospital and Monash University, Melbourne, Australia

Although time-limited anti-CD20 antibody + venetoclax is highly effective for patients (pts) with chronic lymphocytic leukaemia (CLL), there are minimal data regarding its effectiveness among Bruton tyrosine kinase inhibitor (BTKi)-exposed pts.

Aim: Describe the clinico-pathologic characteristics and outcomes of a cohort of pts treated with venetoclax-based therapy for CLL after prior BTKi-exposure.

Method: We retrospectively reviewed 49 such pts consecutively treated at the Royal Melbourne Hospital and Peter MacCallum Cancer Centre, the Princess Alexandra Hospital and Royal North Shore Hospital between June 2011 and Feb 2023. Baseline clinico-pathologic variables, treatment, response and outcome data were collected. The Kaplan-Meier method was used to estimate progression-free survival (PFS) and overall survival (OS).

Results: The median age was 70 (range, 47-86) years and the median number of prior therapies, including BTKi-containing regimen, was 2 (range 1-7). 86% of pts were chemoimmunotherapy-exposed. Median time to disease progression after initial BTKi-containing therapy was 32 (range 1-91) months. At the time of venetoclax-based therapy, most pts had high-risk disease genetics: IGHV unmutated (20/23; 87%), complex karyoptype (16/22; 73%), del(17p) and/or *TP53*-mutated (27/40; 68%). Thirty-four (69%) pts received venetoclax-rituximab intended as time-limited therapy; 15 (31%) received continuous venetoclax monotherapy. Among 37 pts evaluated for response, the objective response rate was 76% (complete response rate: 54%). The median PFS after venetoclax initiation was 22.5 (95%CI 9.2-28.9) months and the median OS was 35.9 (95%CI 21.9-NE) months (Fig 1A). The median PFS for pts receiving continuous venetoclax-monotherapy was 10.5 (95%CI 1.1 – 28.9) months and 25.9 (95%CI 9-42.2) for those receiving venetoclax-rituximab (*p*=0.470) (Fig 1B).



Conclusion: In this heavily pre-treated cohort of mostly chemoimmunotherapy-exposed pts, enriched for high-risk genetics, venetoclax-based therapy was effective against BTKi-exposed CLL; however progressive disease during or shortly after fixed-duration therapy is common. The implications of these data for pts who progress after first-line BTKi are unclear.

A novel analysis of histone methylation marks in CART cell clinical products and T cell substrates identifies new transcription factors and associations with product expansion.

Fiorenza S[1], Zheng Y[2], Sarthy J[3], Purushe J[2], Sheih A[2], Kimble E[2], Kirchmeier D[2], Gauthier J[2], Hirayama A[2], Moloney D[2], Henikoff S[2], Turtle C[1]
[1]University Of Sydney, Camperdown, Australia, [2]Fred Hutchinson Cancer Centre, Seattle, USA, [3]Seattle Children's Hospital, Seattle, USA

Aim: Chimeric antigen receptor (CART) therapy has heralded a revolution in blood cancer immunotherapy. Circulating human T cell naïve (N), central memory (CM) and effector memory (EM) subsets form the substrate for CART cell infusion products (IP) and perform differently in preclinical and clinical studies. Yet identifying genetic differences between these subsets by RNA-sequencing (RNA-seq) has been challenging. This study aims to address the shortcomings of RNA-seq and parse differences in the distinct capabilities of N-, CM- and EM-derived CARTs from HDs and PTs using epigenomic analyses.

Method: We compared RNA-seq to Cleavage Under Targets & Release Using Nuclease (CUT&RUN) analyses of H3K4me2 (K4) and H3K27me3 (K27) histone methylation marks (HMM) in subset-derived CARTs from human healthy donors (HDs) and patients (PT) with aggressive B cell lymphoma from a phase 1/2 clinical trial (NCT01865617).

Results: HMM more accurately distinguished T cell subsets than RNA-seq. HMM of K4 and K27 enriched for 33-fold and 44-fold more genes than RNA-seq when comparing CM- vs EM-derived CART cells, including transcription factors associated with T cell differentiation, exhaustion and lipid metabolism (e.g. KLF7, ZEB2, LEF1, POU2F1, RELA, NFKB, MYC, STAT1, SREBF1), none of which were identified by RNA-seq. Comparison of HD and PT CART cells derived from a CM-enriched IP showed that HMM analyses identified T cell exhaustion-associated transcription factors (e.g. TOX, NR4A3, MYB, KLF2, ETV1) that were not seen by RNA-seq. We further show that HMM but not RNA-seq can identify differences between high and low expanding CART cells following transfer into patients with the same disease, who received the same dose of CART IP, and all showed a complete response to therapy. In this highly select group of patients, we identify the transcription factor, KLF7, and its epigenomic regulome, as being associated with CART cell expansion in a clinical trial.

Conclusion: This work shows that low-cost, high-throughput, high-efficiency epigenomic analyses can distinguish differences between adoptive T cell therapy substrate and products that forecast IP behaviour, identify established and potential new transcription factors that may improve subsequent generations of CART cell therapy.

Targeted next generation sequencing (NGS) of circulating tumour DNA (ctDNA) in patients with aggressive B-cell lymphoma - from diagnosis to transformation at relapse.

Waltham M[2], Wong J[1], Polekhina G[2], Potezny T[1], Gregory G[1,2], Opat S[1,2], **Shortt J[1,2]**
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia

Aim: NGS of ctDNA provides an organismal snapshot of tumour mutations. We aimed to develop a ctDNA assay for subclassification of aggressive B-cell lymphoma (aBL), dynamic response assessment, minimal residual disease (MRD) detection and tracking clonal evolution. Prespecified performance characteristics included capacity to detect point mutation/indels and genome wide copy number variations (CNV).

Method: aBL patients at high risk of treatment failure were prospectively recruited. Baseline and longitudinal plasma sampling occurred at time-points correlating with interim and end of treatment (EOT) PET assessments and during post-remission monitoring. The utility of cycle 1, day 8 ctDNA analysis was specifically explored. NGS libraries were constructed using Agilent chemistry incorporating unique molecular indexing (UMI). A bespoke bait set for 42 genes enriched for targets of interest. Illumina sequencing was performed and data processed via an in-house bioinformatic pipeline.

Results: 20 subjects were recruited over 2.5 years. The majority (18/20) were treatment-naïve receiving RCHOP-like therapy. The EOT overall response rate was 70%, with 3 early deaths and 3 late relapses (2 Hodgkin transformations). Median follow up was 49 months with median PFS/OS not reached. Spiking experiments determined assay sensitivity of 0.2%. Somatic mutations (range: 1-37) were detected at baseline in 19/20 patients. The frequency and distribution of impacted genes was consistent with prior reports and sufficient to infer cell-of-origin, 'druggable hotspots' and the potential presence of *cMYC* and *BCL2* rearrangements. Marked CNVs were identified in a subset of patients independent of ctDNA levels. Clearance of plasma variants by day 8 correlated with clinical responses. MRD positive CRs associated with late relapse, including Hodgkin transformations with shared and emerging variants.

Conclusion: Capture-based NGS of UMI barcoded ctDNA permits molecular subclassification, dynamic risk stratification and tracking of clonal evolution from MRD positive CR to relapse/transformation in aBL. Clinical responses may be predicted as early as day 8.
This research was supported by Amgen. The company had no role in analysing the data or preparing the abstract.

Dissecting immune dysregulation in acquired Bone Marrow Failure Syndromes to identify new therapeutic leads

Koldej R[1,2], Prabahran A[1,2,3], Tan C[4,5], Ludford-Menting M[1,2], Morgan H[1,2], Holzwart N[1,2], Davis M[4,5,6], Ritchie D[1,2,3]

[1]ACRF Translational Research Laboratory, Royal Melbourne Hospital, Melbourne, Australia, [2]Department of Medicine, MDHS, University of Melbourne, Melbourne, Australia, [3]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia, [4]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [5]Department of Medical Biology, MDHS, University of Melbourne, Melbourne, Australia, [6]Department of Clinical Pathology, MDHS, University of Melbourne, Melbourne, Australia

Aim: Poor Graft Function (PGF), manifested by multilineage cytopenias and complete donor chimerism post allogeneic stem cell transplantation (alloSCT), and acquired Aplastic Anaemia (AA) are immune mediated acquired bone marrow (BM) failure syndromes with a similar clinical presentation. In this study, we explored if these conditions share a common BM immunopathology.

Method: Spatial proteomics utilising the NanoString GeoMX platform was used to compare the immunobiology of the BM microenvironment in primary patient samples and identify common mechanisms of immune dysregulation.

Results: Archival BM trephines from patients exhibited significant changes in the expression of VISTA, ARG1 and B7-H3 compared to normal controls. Increased CD163 and CD14 expression suggested monocyte/macrophage skewing which, combined with dysregulation of STING and VISTA, are indicative of an environment of reduced immunoregulation resulting in the profound suppression of haematopoiesis in these 2 conditions. Diagnostic AA samples exhibited a greater degree of dysregulation than PGF suggesting that these diseases represent a spectrum of immune dysregulation. Unexpectedly, analysis of samples from patients with good graft function post alloSCT demonstrated significant changes in the immune microenvironment compared to normal controls, with downregulation of CD44, STING, VISTA and ARG1 suggesting that recovery of multilineage haematopoiesis post alloSCT does not reflect recovery of immune function and may prime patients for the development PGF upon further inflammatory insult.

Conclusion: The BM immune dysregulation in AA/PGF is consistent with a chronic inflammatory response of both myeloid and lymphoid BM resident immune lineages in the absence of critical immune regulators. This study has wide ranging implications for the development of new treatments for aBMFS, suggesting that changing the focus from T cell modifying therapies to those that modulate chronic inflammatory responses across myeloid and lymphoid linages will reduce stem cell suppression and result in restoration of haematopoiesis.

Sensitive measurement of residual disease in granulocytes and T cells strongly predicts relapse in chronic myeloid leukaemia patients stopping therapy

Pagani I[1,2,3], Naranie S[1,2,3,4,5,6], Dang P[1], Saunders V[1], Tolland M[1,2], Grose R[1], Kok C[1,2], Braley J[5], Altamura H[5], Yeung D[1,2,3,4], Branford S[5,6,7], Yong A[8,9], Hughes T[1,2,3,4], Ross D[1,2,3,4,6,10]
[1]Precision Cancer Medicine Theme, Blood Cancer Program, Chronic Myeloid Leukaemia Research Group, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, Australia, [2]School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia, [3]Australasian Leukaemia and Lymphoma Group, Melbourne, Australia, [4]Department of Haematology and Bone Marrow Transplantation, Royal Adelaide Hospital, Adelaide, Australia, [5]Genetic and Molecular Pathology, SA Pathology, Adelaide, Australia, [6]Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia, [7]Clinical & Health Sciences, University of South Australia, Adelaide, Australia, [8]Department of Haematology, Royal Perth Hospital, Perth, Western Australia, Perth, Australia, [9]The University of Western Australia Medical School, Perth, Western Australia, Perth, Australia, [10]Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Adelaide, Australia

Aim: About 50% of Chronic Myeloid Leukaemia (CML) patients who are eligible for Treatment-Free Remission (TFR), relapse after cessation of Tyrosine Kinase Inhibitors (TKIs). There is therefore a need for accurate predictors of TFR. Sensitive detection of residual disease in leukocytes is not highly discriminatory, because leukocytes comprise a mixture of granulocytic and lymphoid lineages, associated with different relapse risk. In this study we tested whether lineage-specific measurement of residual disease is a more accurate predictor of relapse.

Method: Peripheral blood was collected from 40 CML patients in deep-molecular-response (*BCR::ABL1* ≤ 0.01%) who were planning to stop any TKI for the first time. Patient-specific DNA nested Q-PCR (detection limit $10^{-6.2}$) was performed on sorted granulocytes, monocytes, B-,T-, and NK cells. Relapse was defined as loss of major-molecular-response (*BCR::ABL1* >0.1%). Kaplan-Meier analysis based on *BCR::ABL1* DNA positivity(+)/negativity(-) was performed to calculate the probability of relapse.

Results: Comparing patients who subsequently relapsed (58%) with those who maintained TFR (45%), *BCR::ABL1* DNA was present at higher levels in leukocytes (p=0.032), granulocytes (p=0.003) and T cells (p=0.009), but not in monocytes, B cells, or NK cells. Combining granulocytes and T cells we defined three groups with differing probability of relapse: granulocytes+ (n=9) 100%; granulocytes-/T cells+ (n=16) 67%; granulocytes- /T cells- (n=14) 25% (p<0.0001). In multivariate Cox regression analysis, granulocytes remained the only prognostic factor (p=0.002 at 60 months, concordance=0.752), whilst T cells lost significance (p=0.108), indicating that they are likely covariates, as would be expected if they originate from a common precursor population.

Conclusion: These data support the critical importance of a lineage-specific assessment of residual disease: granulocytes-/T cells- could be seen as a 'green light' for selecting patients who can proceed with a TFR attempt, and granulocytes+ as "red light" for patients who should need a different therapeutic approach.

Clonal haematopoiesis mutants detected at the time of stopping therapy are associated with achievement of treatment-free remission in patients with chronic myeloid leukaemia

Fernandes A[1,2,3], Shahrin N[3], Wadham C[3,5], Shanmuganathan N[1,2,3,4,5], Lin M[3], Toubia J[3,5], Yong A[2], Ross D[3,4], Pagani I[1,2], Hughes T[1,2,4], Branford S[1,3,5]

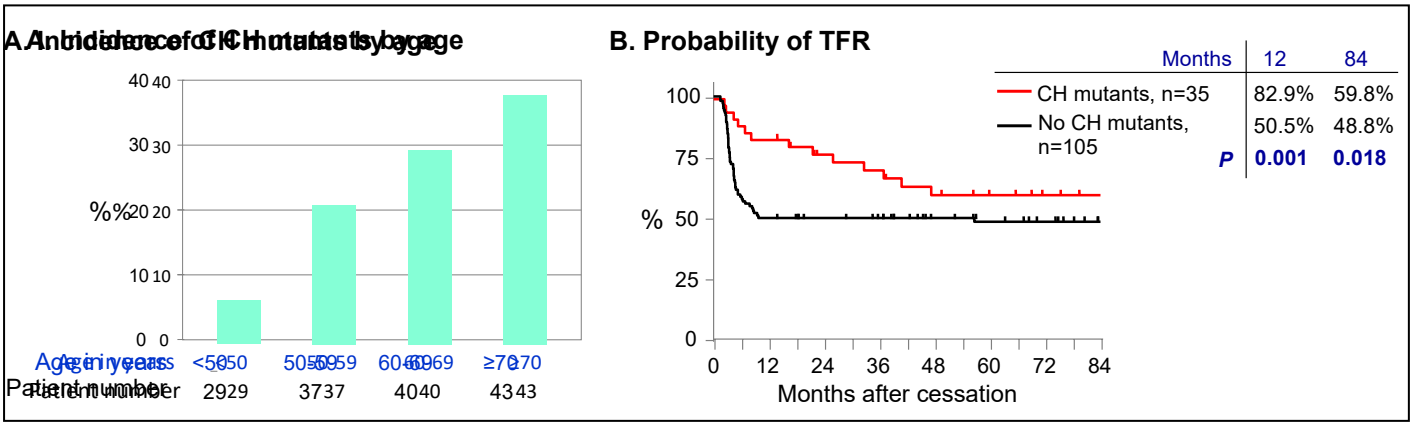
[1]School of Medicine, Faculty of Health and Medical Science,University Of Adelaide, Adelaide, Australia, [2]South Australian Health and Medical Research Institute, Adelaide, Australia, [3]Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, Australia , [4]Department of Haematology, Royal Adelaide Hospital and SA Pathology, Adelaide , Australia, [5]School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia

Aim: Chronic myeloid leukaemia (CML) is hallmarked by the *BCR::ABL1* fusion and is treated with BCR::ABL1 inhibitors. These may be lifelong and associated with side effects. Treatment-free remission (TFR) is a goal wherein ~25% of patients can stop therapy after a sustained deep molecular response without relapse. Relapse occurs in ~50% of all patients who stop therapy. We aimed to identify predictors of relapse. We hypothesised that age-related clonal haematopoiesis (CH) mutations in non-leukaemic cells could influence TFR. These mutants may confer a proliferative advantage and outcompete an emerging residual leukaemic clone after drug cessation.

Method: CH status of 140 CML patients who stopped treatment was determined. Targeted next-generation sequencing was performed on RNA collected at treatment cessation. CH was defined as mutations with VAF ≥2% and/or multiple mutants with a total VAF ≥2%. The probability of TFR was calculated using Kaplan-Meier analysis.

Results: CH mutants were detected in 25% of patients, correlated with increasing age (Figure A) and most frequently occurred in *TET2* and *DNMT3A*. CH was associated with TFR; 83% and 60% of patients with CH were in TFR at 12 and 84 months after cessation, respectively, compared to 51% and 49% of patients without CH (P=0.001 and P=0.018, Figure B). Interestingly, patients with CH were at a greater risk of late (>12 months) relapse. The probability of late relapse by 84 months was 28% for patients with CH compared with 3.3% for patients without CH,P=0.001.

Conclusion: CH was associated with higher rates of TFR at 12 months, but higher risk of late relapse. Competition between CH mutants and an emerging residual leukaemic clone could delay relapse. Novel therapeutic intervention may be warranted to reduce the risk of late relapse for patients with CH. Patients without CH who maintained TFR at 12 months had a reassuringly low risk of late relapse.



Real-World Outcomes of Elderly Patients with Myelodysplastic Syndromes/Low Blast Count Acute Myeloid Leukaemia Treated with Azacitidine: A Single Centre Retrospective Study

Mahaliyana T[1], Motum P[2,3,4], Getta B[2,4]
[1]South West Sydney Local Health District, Bankstown, Australia, [2]Haematology Department, Liverpool Hospital, Liverpool, Australia, [3]New South Wales Health Pathology, Liverpool, Australia, [4]University of New South Wales, Randwick, Australia

Aim: Myelodysplastic Syndromes (MDS), Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN) and Acute Myeloid Leukaemia (AML) are malignant disorders of aging. Literature shows IPSS-R, Charlson Comorbidity Index (CCI), Lactate Dehydrogenase (LDH), frailty have prognostic significance in Azacitidine-treated patients with myeloid disorders[i ii iii iv].

We report our outcomes in patients aged ≥65 years with myeloid disorders treated with azacitidine and prognostic factors identified.

Method: 72 azacitidine-treated patients aged ≥65 years – 42 with MDS or MDS/MPN, 30 with AML – diagnosed 2010-2019 were identified from our Local Health District myeloid database. Demographics, Charlson Comorbidity Index (CCI), disease-specific biomarkers (IPSS-R, LDH, blast count), organ function/frailty-related biomarkers (bilirubin, eGFR, albumin, CRP, BMI) were extracted from the medical record. Statistical analysis was performed using SPSS.

Results: Elderly patients treated with azacitidine had median age of 76 years (range 65-91 years) with 1.8:1 male/female ratio, median CCI was 6, 56% of patients had Very High IPSS-R scores, similar to previous retrospective cohort studies.

Patients with AML had lower bilirubin, lower albumin and higher CCI than those with MDS or MDS/MPN. Other characteristics were not significantly different.

Median OS in the MDS + MDS/MPN cohort was 12.7 months, 71.2 months in the Intermediate Risk IPSS-R category, 12.8 months in the High Risk category, 12.6 months in the Very High Risk category. Median OS in AML was 8.2 months.

Univariate survival analysis showed IPSS-R to be the only statistically significant prognostic variable.

Conclusion: Azacitidine-treated elderly patients with myeloid disorders in SWSLHD had similar characteristics and median OS to other retrospective studies[v]. Patients with AML had more comorbidities and organ dysfunction than those with MDS, possibly reflecting expected changes with disease transformation. IPSS-R was the only prognostic variable, however low patient numbers may have obscured identification of other factors.

Leukemic transformation STEMming from myeloproliferative neoplasm: Resolving the loss of stem cell heterogeneity

Grabek J[1,2], Straube J[1,2], Bywater M[1,2], Ross D[4], Lane S[1,2,3]

[1]Queensland Institute Of Medical Research, Herston, Australia, [2]University of Queensland, Brisbane, Australia, [3]Cancer Services, Royal Brisbane and Women's Hospital, Herston, Australia, [4]South Australian Cancer Research Biobank, Royal Adelaide Hospital, Adelaide, , Adelaide, Australia

Aim: The acquisition of mutations in chronic phase MPN haematopoietic stem and progenitor cells (HSPC) is a key driver of leukemic transformation. Through the development of a novel single cell analysis pipeline, we have been able to integrate the mutational profile of single cells with transcript expression analysis. We hypothesise that mutational acquisition within the JAK2 V617F mutant stem cells of chronic phase MPN leads to loss of heterogeneity in the HSPC compartment and consequently leukemic transformation.

Method: To determine the effect of gene mutations within the MPN HSPC compartment we examined 12 primary human samples from patients with chronic phase MPN and post MPN leukemia, including 2 matched chronic and leukemic samples. HSPCs were isolated from bone marrow by fluorescence activated cell sorting (FACS) for CD34+ cells with single cells purified and analysed with 10X chromium 3' barcoding. Transcript expression analysis was performed using Illumina short-read sequencing and concurrent mutational profiling was Oxford Nanopore Technology (ONT) long read sequencing. Specifically, single cell barcoded cDNA was enriched for transcripts from 30 genes relevant to MPN disease progression.

Results: We first identified a transcriptional heterogeneity within normal CD34+ cells. Next, we identified that JAK2 V617F mutations increase megakaryocyte and erythroid bias within HSPC of chronic phase MPN sample, however there was preserved transcriptional heterogeneity within these HSPCs. We next examined post-MPN AML samples. Secondary genetic mutations within the JAK2 V617F HSPC population such as IDH2, IDH1, SRSF2, TET2 and TP53 were present post-MPN leukaemia samples and these were associated with a loss of heterogeneity and dominance of a single cell type-specific transcriptional signature. This identity of the cell type-specific transcriptional signature was influenced by individual secondary mutations, with a TP53 mutation leading to a megakaryocyte-erythroid progenitor-like signature compared to an IDH2 mutation leading to a multipotent progenitor-like signature.

Conclusions: We have developed a novel pipeline for integrating transcript expression and mutation profiling for in single cells in MPN samples. Within JAK2 V617F mutant HSPC compartment, we identified secondary genetic lesions that drive loss of transcriptional heterogeneity, a dominant progenitor population and progression to acute leukaemia.

Long road to a short diagnosis – detection of a novel germline TERT variant underlying a complex multisystem telomere biology disorder.

Nagarethinam M[1], Zantomio D[1], Bryan T[2], Fox L[1,3]
[1]Clinical Haematology Department Austin Health, Melbourne, Australia, [2]Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia, Westmead, Australia, [3]Molecular Haematology Department, Peter MacCallum Cancer Centre, Melbourne, Australia

Telomeres are DNA-protein structures at chromosome ends that maintain chromosome stability; their length affects cell replicative potential and senescence. A constellation of bone marrow failure (BMF), pulmonary fibrosis, liver cirrhosis and premature greying is suggestive of a telomere biology disorder (TBD), however incomplete penetrance results in highly variable manifestations.[1]

We report a case of a 30-year-old male who was initially found to have interstitial lung disease with profound orthodeoxia of unclear cause at the age of 21 for which he underwent bilateral lung transplantation. He was also found to have non cirrhotic portal hypertension of unclear aetiology and developed progressive hepatic and renal failure. He underwent simultaneous liver and kidney transplantation a few years later. Prior to the solid organ transplants, a bone marrow biopsy was performed to investigate severe pancytopenia which revealed a markedly hypocellular marrow with marked trilineage hypoplasia. An inherited BMF syndrome was suspected, and a diagnosis of TBD was established by detection of telomere lengths less than the 1st percentile for his age.

Genetic testing revealed a novel heterozygous **TERT variant (NM_198253.2c.3063_3064delinsAT;p.(Phe1021_His1022delinsLeuTyr)** which was initially categorized as a variant of uncertain significance. This variant is unreported in healthy population databases and is located within the C-terminal extension domain of the TERT protein, where pathogenic variants have previously been reported. Functional studies determined that the observed variant resulted in significantly reduced telomerase activity and a modest effect on DNA binding. The *TERT* variant was deemed causative of the severe multisystem disease in the patient.

The combined kidney and liver transplant was complicated by low-grade CMV viraemia, worsening cytopenia and mixed rejection. Subsequently, the patient's cytopenia's improved but macrocytosis remained and he is now having ongoing haematological monitoring.

TBDs are rare, inherited, progressive, and multi-system disease of aberrant telomere maintenance. Significant morbidity and mortality arise from bone marrow failure, haematological malignancies, solid tumours, pulmonary fibrosis, and liver diseases[2].

References :

- Kam MLW, Nguyen TTT, Ngeow JYY. Telomere biology disorders. NPJ Genom Med. 2021 May 28;6(1):36. doi: 10.1038/s41525-021-00198-5. PMID: 34050178; PMCID: PMC8163767.
1. Sharon A. Savage; Dyskeratosis congenita and telomere biology disorders. *Hematology Am Soc Hematol Educ Program* 2022; 2022 (1): 637–648. doi: https://doi.org/10.1182/hematology.2022000394

Improvement in CAR-T outcomes over time – lessons from the UK experience

Boyle S[1], Roddie C[2], Menne T[3], Norman J[4], Gibb A[5], Lugthart S[6], Chaganti S[7], Gonzalez Arias C[8], Jones C[9], Latif A[10], Uttenthall B[11], Seymour F[12], Sanderson R[1], Kuhnl A[1]

[1]King's College Hospital, London, , United Kingdom, [2]University College London Hospitals, , United Kingdom, [3]Freeman Hospital, Newcastle, , United Kingdom, [4]Manchester Royal Infirmary, , United Kingdom, [5]The Christie Hospital, Manchester, , United Kingdom, [6]University Hospitals Bristol and Weston, Bristol, , United Kingdom, [7]Queen Elizabeth Hospital, Birmingham, , United Kingdom, [8]Royal Marsden Hospital, London, , United Kingdom, [9]University Hospital of Wales, Cardiff, , United Kingdom, [10]Queen Elizabeth II Hospital, Glasgow, , United Kingdom, [11]Cambridge University Teaching Hospital, Cambridge, , United Kingdom, [12]Leeds Teaching Hospitals, Leeds, , United Kingdom

Aim: CAR-T therapy is now standard of care in 3rd line relapsed/refractory Large B-cell Lymphoma (r/r LBCL) and is challenging established treatments earlier in the treatment paradigm. This is likely to lead to a substantial increase in use of the commercial products in Australia over the coming years, with an imperative that we use the treatment in the most effective manner possible for our patients. We present data from the national UK experience, highlighting substantial improvement in patient outcomes over time in a real-world cohort of >700 patients, and important shifts in management that are relevant to Australian practice.

Method: We gathered data on the first 726 UK patients approved for commercial axicabtagene ciloleucel (axi-cel) or tisagenlecleucel (tisa-cel) for r/r LBCL and split the patients into two cohorts– those approved for treatment in year one (Dec-2018 to Dec-2019: Era-1) and those approved after this (Jan-2020 to Jun-2022: Era-2). We performed chi-squared testing to compare baseline factors, Kaplan Meier Log-Rank test to compare Overall Survival (OS) and Progression-Free Survival (PFS) and Cox Regression to identify factors associated with improved outcomes.

Results: We observed a substantial improvement in CAR-T patient outcomes after the first year of use in the UK, with 1-yr-PFS/OS rising from 25%/39% in Era-1 to 44%/59% in Era-2 (both p<0.001, Figs 1&2), with Era-2 outcomes now similar to the pivotal trials. We saw substantial changes in bridging therapy, increased axi-cel use and more pro-active toxicity management over time and identified improved bridging as an important predictor of OS/PFS (Table).

Conclusion: These data provide evidence of improved CAR-T outcomes associated with specific practice changes from a national cohort of patients treated in a similar healthcare environment to Australia and should be considered by Australian centres aiming to evolve and improve their CAR-T service.

Fig 1: PFS of infused patients by treatment era

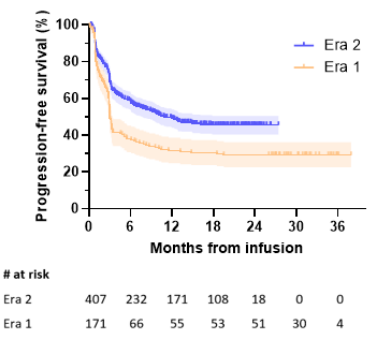
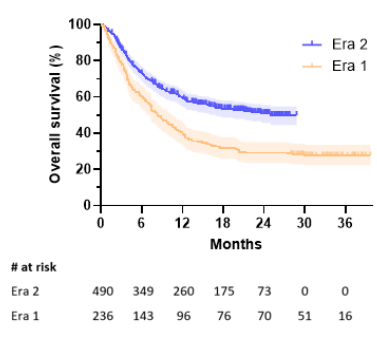


Fig 2: OS (ITT) from CAR-T approval date by Era



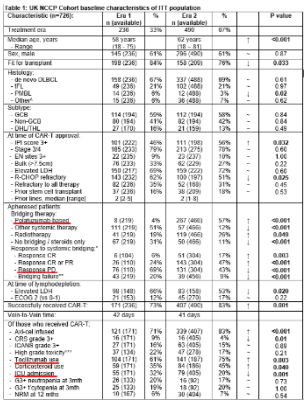


Table 1: UK NCCP Cohort baseline characteristics of ITT population

Characteristic (n=726):	Era 1 n (available)	%	Era 2 n (available)	%		P value
Treatment era	236	33%	490	67%		
Median age, years (Range)	58 years (18 – 75)		62 years (18 – 81)		↑	<0.001
Sex, male	145 (236)	61%	296 (490)	61%	~	0.87
ECOG transplant	198 (236)	84%	158 (209)	76%	↓	0.033
Histology:						
- de novo DLBCL	158 (236)	67%	337 (488)	69%	~	0.61
- tFL	49 (236)	21%	102 (488)	21%	~	0.87
- PMBL	14 (236)	6%	12 (488)	3%	↓	0.02
- Other*	15 (236)	6%	36 (488)	7%	~	0.62
Subtype:						
- GCB	114 (194)	59%	112 (194)	58%	~	0.84
- Non-GCB	80 (194)	41%	82 (194)	42%	~	0.84
- DHL/THL	27 (170)	16%	21 (159)	13%	~	0.45
At time of CAR T approval:						
- IPI score 3+	161 (222)	46%	111 (198)	56%	↑	0.032
- Stage 3/4	185 (233)	79%	213 (275)	78%	~	0.66
- EN sites 2+	22 (235)	9%	21 (237)	10%	~	1.00
- Bulk (>7.5cm)	76 (233)	33%	62 (229)	27%	~	0.22
- Elevated LDH	158 (217)	69%	159 (222)	72%	~	0.66
- R-CHOP refractory	143 (232)	62%	109 (197)	51%	↓	0.025
- Refractory to all therapy	62 (236)	25%	52 (188)	31%	~	0.45
- Prior stem cell transplant	37 (236)	16%	38 (209)	18%	~	0.53
- Prior lines, median (range)	2 (2-5)		2 (1-8)			
Apheresed patients:						
Bridging therapy:						
- Polatuzumab based	8 (219)	4%	287 (466)	57%	↑	<0.001
- Other systemic therapy	111 (219)	51%	57 (466)	12%	↓	<0.001
- Radiotherapy	41 (219)	19%	119 (466)	26%	↑	0.049
- No bridging / steroids only	67 (219)	31%	50 (466)	11%	↓	<0.001
Response to systemic bridging:*						
- Response CR	6 (104)	6%	51 (304)	17%	↑	0.003
- Response CR or PR	76 (110)	24%	143 (304)	47%	↑	<0.001
- Response PD	76 (110)	69%	131 (304)	43%	↓	<0.001
- Bridging failure**	43 (219)	20%	39 (456)	9%	↓	<0.001
At time of lymphodepletion:						
- Elevated LDH	98 (148)	66%	82 (158)	52%	↓	0.020
- ECOG 2 (vs 0-1)	31 (153)	12%	45 (270)	17%	~	0.22
Successfully received CAR-T:	171 (236)	73%	407 (490)	83%	↑	0.001
Vein-to-Vein time:	42 days		41 days			
Of those who received CAR-T:						
- Axi-cel infused	121 (171)	71%	339 (407)	83%	↑	<0.001
- CRS grade 3+	16 (171)	9%	16 (405)	4%	↓	0.01
- ICANS grade 3+	27 (171)	16%	63 (405)	15%	~	0.88
- High grade toxicity***	37 (134)	22%	47 (278)	17%	~	0.21
- Tocilizumab use	104 (171)	61%	141 (197)	75%	↑	0.003
- Corticosteroid use	59 (171)	35%	84 (198)	45%	↑	0.040
- ICU admission	55 (171)	32%	79 (405)	20%	↓	0.001
- G3+ neutropenia at 3mth	26 (133)	20%	16 (92)	17%	~	0.73
- G3+ thrombocytopenia at 3mth	25 (122)	19%	18 (92)	20%	~	1.00
- NRM at 12 mths	10 (167)	6%	30 (404)	7%	~	0.64

Single-institution outcomes of standard-of-care chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory (R/R) large B-cell lymphoma (LBCL) in third-line or beyond: an Australian real-world experience

Scott H[1,2], **Dowling M[1,2]**, van der Linde S[1,2], Panozzo B[1,2], Minson A[1,2], Hofman M[1], Kuzich J[1,2], O'Leary N[1,2], Rodríguez Díaz N[1,2], Saghebi J[1], Wong A[1], Chee L[1,2], Cummins K[1,2], Thompson P[1,2], Tam C[3], Bajel A[1,2], Ritchie D[1,2], Seymour J[1,2], Anderson M[1,2], Dickinson M[1,2], Harrison S[1,2]
[1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Royal Melbourne Hospital, Melbourne, Australia, [3]Alfred Health, Melbourne, Australia

Aim: CAR T-cell therapy has been available in Australia since February 2020 as a standard-of-care publicly-funded treatment for fit patients with R/R LBCL after two or more prior lines of therapy. We evaluated real-world single centre Australian outcomes for patients receiving axicabtagene ciloleucel (axi-cel) or tisagenlecleucel (tisa-cel) for this indication.

Method: Data were collected retrospectively for all patients with R/R LBCL that underwent leukapheresis with intent to receive tisa-cel or axi-cel at the Peter MacCallum Cancer Centre.

Results: 114 patients underwent leukapheresis with intent to proceed to CAR T-cell therapy (59 tisa-cel, 55 axi-cel), of which 100 (88%) subsequently received the CAR T-cell infusion (50 tisa-cel, 50 axi-cel). Baseline characteristics are shown in Fig. 1A. The best complete response rate (CRR) was 71% for all infused patients, 70% for tisa-cel and 72% for axi-cel infused patients. Progression-free survival (PFS) at 12 months was 0.39 (95% CI: 0.29–0.52) for all infused patients, 0.33 (0.22–0.50) for tisa-cel and 0.50 (0.36–0.69) for axi-cel infused patients (Fig. 1B). On intention-to-treat (ITT) analysis including all leukapheresed patients, 12-month PFS was 0.34 (0.25–0.46) for all CAR-T-intended patients, 0.28 (0.18–0.43) for tisa-cel-intended patients and 0.45 (0.32–0.64) for axi-cel-intended patients. Grade 3 cytokine release syndrome occurred in 5% (6% tisa-cel, 4% axi-cel), and grade 3-4 immune-effector cell associated neurotoxicity syndrome occurred in 9% of patients (2% tisa-cel, 16% axi-cel). Outcomes were stratified by published baseline clinical and laboratory risk factors, such as elevated LDH (Fig. 1C), tumour burden, extranodal disease and refractoriness to prior therapies.

Conclusion: Our single-institution outcomes with CAR T-cell therapy for LBCL in third line or beyond in the Australian real-world context compare favourably with pivotal trials, with a low incidence of severe toxicities and durable responses despite the presence of one or more baseline risk factors.

A

	All (N=100)	Tisa-cel (N=50)	Axi-cel (N=50)
Age			
med (min - max) (years)	64 (31 - 80)	66 (43 - 77)	62 (31 - 80)
Sex			
Male	57 (57%)	31 (62%)	26 (52%)
Female	43 (43%)	19 (38%)	24 (48%)
ECOG			
0	63 (63%)	33 (66%)	30 (60%)
1	37 (37%)	17 (34%)	20 (40%)
Histology			
HGBL	5 (5%)	1 (2%)	4 (8%)
DLBCL	69 (69%)	33 (66%)	36 (72%)
tFL	14 (14%)	10 (20%)	4 (8%)
tMZL/tWM	4 (4%)	2 (4%)	2 (4%)
PMBCL	3 (3%)	1 (2%)	2 (4%)
Richters	3 (3%)	1 (2%)	2 (4%)
No. prior lines			
2	63 (63%)	30 (60%)	33 (66%)
3	22 (22%)	13 (26%)	9 (18%)
4+	15 (15%)	7 (14%)	8 (16%)
Prior AutoSCT			
No	65 (65%)	26 (52%)	39 (78%)
Yes	35 (35%)	24 (48%)	11 (22%)
Primary refractory or relapse <12M			
No	31 (31%)	17 (34%)	14 (28%)
Yes	67 (67%)	31 (62%)	36 (72%)
Response to most recent therapy			
Relapsed	40 (40%)	26 (52%)	14 (28%)
Refractory	60 (60%)	24 (48%)	36 (72%)
Response to bridging therapy			
No bridging	13 (13%)	12 (24%)	1 (2%)
PR/CR	37 (37%)	19 (38%)	18 (36%)
SD/PD	48 (48%)	17 (34%)	31 (62%)
LDH (pre-LD)			
Normal	62 (62%)	32 (64%)	30 (60%)
Elevated	38 (38%)	18 (36%)	20 (40%)

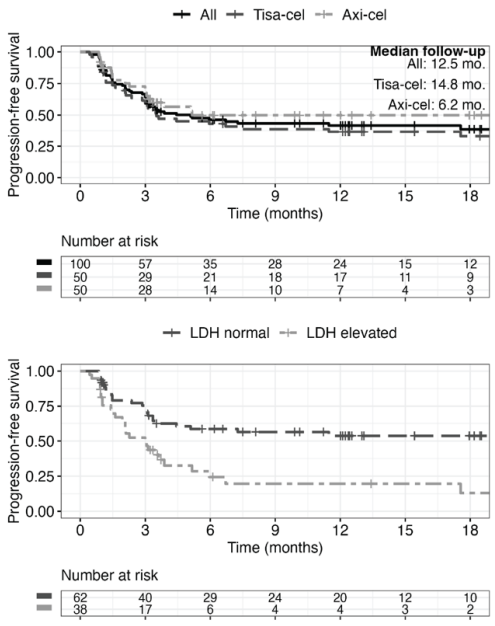


Figure 1: (A) Baseline characteristics. (B) PFS for all patients and stratified by product. (C) PFS stratified by pre-leukapheresis LDH.

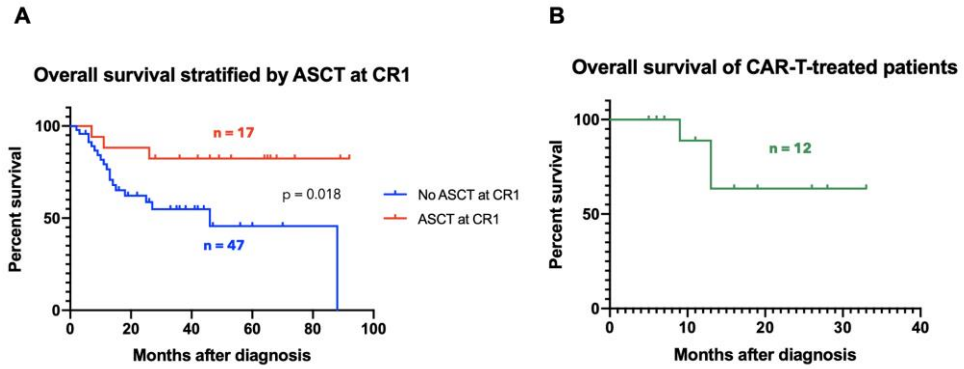
Real-world outcomes of patients with double-hit lymphoma treated from 2013 to 2023 at Royal Melbourne Hospital and Peter MacCallum Cancer Centre

Lin V[1], Bennett R[1], Minson A[1], Seymour J[1], Anderson M[1], Dickinson M[1]
[1]*Department of Clinical Haematology, Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville, Australia*

Aim: Double-hit lymphoma (DHL), defined as high-grade B-cell lymphoma with rearrangement of MYC plus BCL2 and/or BCL6 by FISH (WHO 2016), is associated with inferior prognosis compared with diffuse large B-cell lymphoma. While treatment strategies for DHL have evolved with time, to date, no standard-of-care exists in the upfront or relapsed/refractory (R/R) setting. We aimed to describe our institutional experience treating patients with DHL in the contemporary era.

Method: We retrospectively reviewed 64 consecutive patients with proven DHL treated at Royal Melbourne Hospital and Peter MacCallum Cancer Centre from June 2013 to March 2023. Baseline clinicopathologic variables, treatment, response and outcome data were collected. Kaplan-Meier method was used to estimate overall survival (OS).

Results: Median age at diagnosis was 61.5 (range, 33–98) years. 41 cases arose *de novo*; 23 were transformed from low-grade lymphoma (follicular lymphoma, *n*=21; small lymphocytic lymphoma, *n*=1; MALT lymphoma, *n*=1). Initial treatment consisted of DA-R-EPOCH (*n*=29), R-CHOP (*n*=24), R-CODOX-M/R-IVAC (*n*=3), Pola-R-CHP combination therapy (*n*=2), R-ICE (*n*=2), R-HyperCVAD (*n*=1), G-EPOCH (*n*=1), R-CHOP + tafasitamab (*n*=1), and steroids/cyclophosphamide (*n*=1). 20 received consolidative ASCT (17 in CR1, 3 in CR2); conditioning regimens were CBV (n=17), LACE (n=1), BEAM (n=1), and unknown (n=1). Response to DA-R-EPOCH was superior to R-CHOP with CR rates of 72% vs 58% with no significant benefit in OS (p=0.130). Median OS was 46 months for patients who did not receive consolidative ASCT in CR1 and not reached for those who received consolidative ASCT in CR1 (p=0.018); 5-year survival rate=47%. Of the 12 patients who received CAR-T (3 at first relapse, 9 at subsequent relapse), 2-year survival rate was 63%.



Conclusion: For patients with DHL, initial treatment with R-EPOCH is associated with higher CR rates and consolidative ASCT in CR1 with significantly better OS. CAR-T cell therapy may be an effective salvage option for patients with R/R DHL.

Glofitamab monotherapy induces high complete response rates in patients with heavily pretreated relapsed or refractory mantle cell lymphoma

Dickinson M[1], Phillips T[2], Morschhauser F[3], Bachy E[4], Crump M[5], Trněný M[6], Bartlett N[7], Zaucha J[8], Wrobel T[9], Offner F[10], Humphrey K[11], Lundberg L[12], Relf J[11], Filezac de L'Etang A[12], Carlile D[11], Byrne B[11], Qayum N[11], Carlo-Stella C[13]

[1]Peter Maccallum Cancer Centre, Royal Melbourne Hospital And The University Of Melbourne, Melbourne , Australia, [2]University of Michigan Medical School, Ann Arbour, USA, [3]CHU Lille Service des Maladies du Sang, Lille, France, [4]Hospices Civils de Lyon and Université Claude Bernard, Pierre-Bénite, France, [5]Princess Margaret Hospital, Toronto, Canada, [6]Fac Medicine, Charles University, Prague, Czech Republic, [7]Siteman Cancer Center, Washington University, USA, [8]Medical University of Gdańsk, Gdańsk, Poland, [9]Wroclaw Medical University, Wroclaw, Poland, [10]Dept Hematology Universitair Ziekenhuis, Gent, Begium, [11]Roche Products Ltd,, Welwyn Garden City, United Kingdom, [12]F. Hoffmann-La Roche Ltd, Basel, Swizerland, [13]Humanitas University and IRCCS Humanitas Research Hospital, Milan, Italy

Aim: To report on the durability of response with glofitamab Step-Up Dosing (SUD) in a larger cohort with additional follow-up from a Phase I/II (NCT03075696) study.

Methods: Patients received Obinutuzumab pretreatment (Gpt) 7-days prior to IV-glofitamab. glofitamab SUD was given on C1D8 (2.5mg), C1D15 (10mg) then at the target dose (16mg or 30mg) from C2D1, every 3 weeks for up to 12 cycles. Response rates were assessed by PET-CT.

Results: 37 patients in 2 cohorts received glofitamab SUD after Gpt (1000mg,n=16; 2000mg, n=21) after a median of 3(1-5) prior therapy lines. Most patients (91.9% Ann Arbor Stage III/IV; 32.4% MIPI score ≥6) had prior BTKi (64.9%) or lenalidomide (18.9%) and 62.2% refractory to first and/or last (73.0%) prior therapy.

After 8-months median follow-up (0–19), overall response rate and complete response (CR) were 83.8% and 73.0%, respectively, in combined Gpt cohorts; median time to CR was 51 days. The majority of CR (20/27; 74.1%) were ongoing at data cut-off; an estimated 71.6% of patients in CR remained in response at 9 months. Median duration of response was 12.6 months (95%CI: 10.0–NE); median duration of CR was 10.0 months (95%CI: 4.9–NE.

The most common adverse events (AEs, safety-evaluable n=37) were ASCTC-CRS (75.7%) and neutropenia (40.5%). CRS rates were lower in the 2000mg Gpt (14/21; 66.7%) vs the 1000mg Gpt (14/16; 87.5%) cohort. CRS events in the 2000mg Gpt cohort were all Grade 1–3. All CRS events were manageable and most resolved by data cut-off.

Neurologic AEs (NAEs) occurred in 19 patients (51.4%) and were mostly ofGr 1/2. Ten deaths were reported and considered unrelated to study treatment.

Conclusions: Fixed-duration glofitamab monotherapy after Gpt induced high rates of durable, mostly early CR in heavily pretreated patients with MCL. CRS events were manageable and mostly low grade.

Glofitamab monotherapy in patients with relapsed/refractory Large B-Cell Lymphoma: Extended follow-up and landmark analyses from pivotal phase II study

Dickinson M[1], Falchi L[2], Carlo-Stella C[3], Morschhauser F[4], Hutchings M[5], Bachy E[6], Cartron G[7], Chan C[8], Tani M[9], Martinez-Lopez J[10], Bartlett N[11], Salar A[12], Brody J[13], Lassa S[14], Mulvihill E[15], Lundberg L[15], Relf J[16], Xie Y[17], Bottos A[15], Humphrey K[16]

[1]Peter Maccallum Cancer Centre, Royal Melbourne Hospital And The University Of Melbourne, Melbourne , Australia, [2]Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York, USA, [3]Humanitas University and IRCCS Humanitas Research Hospital, Milan, Italy, [4]Hôpital Claude Huriez and CHU de Lille, Lille, France, [5]Rigshospitalet, Copenhagen, Denmark, [6]Centre Hospitalier Lyon-Sud, Lyon, France, [7]CHU de Montpellier, Montpellier, France, [8]Allegheny Health Network, Pittsburgh, USA, [9]Ospedale Santa Maria delle Croci, Ravenna, Italy, [10]Hospital Universitario 12 de Octubre, Madrid, Spain, [11]Washington University School of Medicine, St Louis, USA, [12]Hospital del Mar, Barcelona, Spain, [13]Tisch Cancer Institute, New York, USA, [14]University of Helsinki and Helsinki University Hospital Comprehensive Cancer Cente, Helsinki, Finland, [15]F. Hoffmann-La Roche Ltd, Basel, Switzerland, Basel, Switzerland, [16]Roche Products Ltd, Welwyn Garden City, UK, [17]F. Hoffmann-La Roche Ltd, Mississauga, Canada

Aim: To present extended follow-up and landmark analysis of complete response (CR) outcomes in R/R LBCL patients treated with glofitamab.

Methods: LBCL Patients with ≥2 prior therapies were pre-treated with 1000mg obinutuzumab, 7 days prior to first IV-glofitamab step-up dose (2.5mg,C1D1) to 30mg (target dose,C2D1). Primary endpoint was CR rate (CRR) by independent review committee (IRC). PFS/OS post-hoc analyses were performed in responders (CR at C3 or end of treatment [EOT]).

Results: 154-patients received ≥1 dose of study treatment. Median prior therapies was 3 (range: 2-7); 85% refractory to their most recent regimen. Median 20.1months (range: 0–32) on study. Investigator-assessed CRR (BOR)=38%(40%- IRC); ORR= 59%(52%-IRC). Most CRs (66%) were ongoing after median follow-up of 18.3months (range: 0–30). Median duration of CR (DoCR)= 24.1-months (95% CI: 19.8–NE); 70% of patients with CR at any time remained in remission at 18months; 18month OS rate= 41% (95% CI: 32.1–49.3). Analyses at 1-year in patients with CR before C3 (PFS/OS :71%/92%) and in patients with a CR at EOT (PFS/OS: 80%/94%) showed that most patients were progression free and alive. In a cohort of 101 patients treated with glofitamab doses <RP2D but ≥10mg (median CR follow-up 31-months, range: 1–49), median DoCR was 30.1-months (95% CI: 5.5–NE); 55% of patients were in remission at cut-off date, confirming the highly durable glofitamab responses.
ASTCT CRS remained the most common adverse event (AE); in 64% of patients mostly Grade (Gr) 1(48%) or 2(12%). Incidence of AEs/serious AEs was comparable with earlier analyses; 1 acute kidney injury (Gr3) and 1 agitation (Gr2), but no glofitamab-related Gr5 AEs newly reported.

Conclusions: Glofitamab continued to demonstrate durable responses, with most patients in CR at EOT still in remission without new AEs. Data support the potential for favourable long-term outcome with glofitamab for R/R LBCL.

Epcoritamab SC induces durable complete remissions in patients with relapsed/refractory diffuse large B-cell lymphoma: long-term results of EPCORE NHL 1

Gasiorowski R[1], Thieblemont C[2], Gasiorowski R[3], Ghesquieres H[4], Jurczak W[5], Clausen M[6], Lugtenburg P[7], Cunningham D[8], Do Y[9], Lewis D[10], Kim T[11], van der Poel M[12], Poon M, Feldman T[14], Linton K[15], Sureda A[16], Hutchings M[17], Stirner M[18], Liu Y[19], Kilavuz N, Sacchi M[19], Cheah C[20]
[1]Concord Hospital, University of Sydney, , Australia, [2]Assistance Publique & Hopitaux de Paris (APHP), Hôpital Saint-Louis, Hémato-oncologie, Université de Paris, , France, [3]University of Michigan Comprehensive Cancer Center, Ann Arbor, USA, [4]Hospices Civils de Lyon, Centre Hospitalier Lyon Sud, , France, [5]MSC National Research Institute of Oncology, , Poland, [6]Vejle Hospital, , Denmark, [7]On behalf of the Lunenburg Lymphoma Phase I/II Consortium-HOVON/LLPC, Erasmus MC Cancer Institute, University Medical Center, Department of Hematology, , Netherlands, [8]The Royal Marsden NHS Foundation Trust, , UK, [9]Keimyung University Dongsan Medical Center, , Republic of Korea, [10]University Hospitals Plymouth NHS Trust, Derriford Hospital, , UK, [11]Seoul National University Hospital, , Republic of Korea, [12]On behalf of the Lunenburg Lymphoma Phase I/II Consortium-HOVON/LLPC, Maastricht, Department of Internal Medicine, Division of Hematology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, , Netherlands, [13]National University Hospital, , Singapore, [14]Hackensack Meridian Health Hackensack University Medical Center, Hackensack, USA, [15]The Christie NHS Foundation Trust and Manchester Cancer Research Centre, , UK, [16]Institut Català d'Oncologia, Hospital Duran i Reynals, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, , Spain, [17]Rigshospitalet, Copenhagen University Hospital, , Denmark, [18]AbbVie, North Chicago, USA, [19]Genmab, Princeton, USA, [20]Sir Charles Gairdner Hospital, , Australia

Aim: Patients with relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) have limited treatment options and poor outcomes. Single-agent epcoritamab, a subcutaneous (SC) CD3xCD20 bispecific antibody, has demonstrated deep and durable responses in patients with R/R large B-cell lymphoma in the EPCORE™ NHL-1 phase 1/2 trial (NCT03625037). Epcoritamab was approved by the US FDA for the treatment of adults with R/R DLBCL, NOS, including DLBCL arising from indolent lymphoma, and HGBCL after ≥2 lines systemic therapy. We present longer-term DLBCL results.

Method: Patients with R/R CD20$^+$ DLBCL and ≥2 prior treatment lines received epcoritamab (step-up doses followed by 48-mg full doses) in 28-d cycles (Cs) until disease progression or unacceptable toxicity: QW, C1–3; Q2W, C4–9; Q4W, C≥10.

Results: As of November 18, 2022, 139 patients (median age, 66 y) had been treated (14% [12/88] double/triple-hit lymphoma by central FISH, 58% primary refractory disease, 38% prior CAR T, median of 3 prior treatment lines [range, 2–11]); 33 (24%) remained on treatment. Overall/complete response (CR) rates were 61.9%/39.6% (median follow-up, 20.0 mo [range, 0.3+ to 28.2]). Median duration of response among complete responders (n=55) and median duration of CR were both 20.8 mo. Median overall survival (OS) was 19.4 mo. Among complete responders, median OS and progression-free survival were not reached. The most common treatment-emergent AEs (TEAEs) of any grade (G) were: CRS, 49.6%; neutropenia, 25.2%; fatigue, 23.7%; pyrexia, 23.7%; and nausea, 23.0%. CRS was mostly low grade (46.0% G1–2) and primarily occurred following the first full dose (median onset, 20 h). Treatment was discontinued due to G1 CRS in 1 patient. Fatal TEAEs were reported in 15 patients; 2 events were considered related (COVID-19 and ICANS).

Conclusion: Longer follow-up reaffirms that single-agent epcoritamab induces durable CRs and impressive long-term outcomes with a manageable safety profile in R/R DLBCL.

Interim 3D volumetric response (3DVR) is associated with better overall survival of patients (pts) with primary CNS lymphoma (PCNSL)

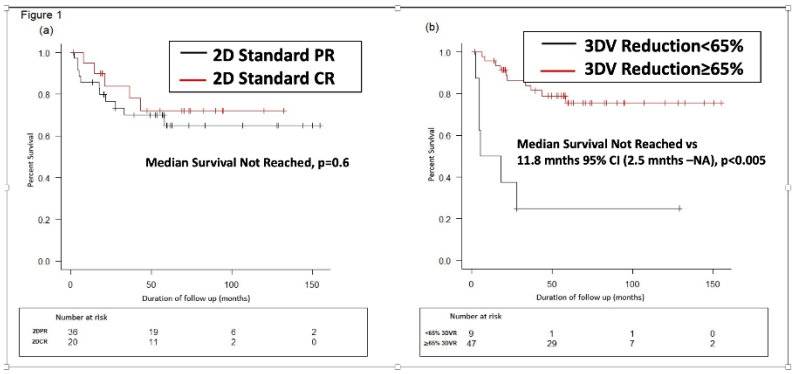
Martynchyk A[1], O'Shaughnessy J[1], Lee S[2], Chong G[1], Agrawal S[1], Tatarczuch M[3], Azryn N[2], Gregory G[3], Churilov L[4], Hawkes E[1]

[1]Olivia Newton-John Cancer and Wellness Centre at Austin Health, Heidelberg, Australia, [2]Austin Health, Molecular Imaging and therapy, Heidelberg, Australia, [3]Monash Health, Melbourne, Australia, [4]University of Melbourne, Melbourne, Australia

Aim: PCNSL outcome risk determination is currently not standardized. Prognostic role of 2Dimensional (2D) response assessment is controversial (1). Reduction of at least 65% in 3DV is prognostic for solid brain tumour outcomes (2). Our aim was to assess the associations of baseline 3DV and 3DVR with survival in pts with PCNSL.

Method: This is a retrospective multicentre study of the impact of MRI-measured 3DV on outcomes in 78 adult PCNSL pts receiving immunochemotherapy between 2009-2021, 60 pts had paired baseline & interim MRIs. 3DV was calculated centrally using MIM Maestro software. 2D response was determined according to standard criteria (3). EZR was used for statistical analysis: Kaplan-Meier survival curve and logrank test - for survival comparisons. Fisher's exact test - to compare pts' characteristics.

Results: Median age was 66.5y (range 22-86); 59.0% were male. All pts received methotrexate-based chemotherapy: R-MPV (54%), MATRIX (13%), other (33%). Median follow-up was 63m, 5y OS was 62.5%. Median baseline 3DV was 11.8 mLs (range 0.23-331.5). Baseline 3DV did not correlate with overall survival (OS). Interim 2D complete response (CR) or partial response (PR) was associated with longer OS compared to stable disease or progression (p=0.01). In pts with 2D interim CR or PR (n=56) there was no OS difference between CR vs PR (p=0.6, Figure 1a), however i3DVR <65% was associated with inferior OS (median OS 11.8m vs not reached; p<0.001, Figure 1). There were no significant differences in age>60y.o., sex, elevated LDH and ECOG 2-4 between groups with CR vs PR, 3DVR □ or <65%.



Conclusion: Association between baseline 3DV and OS was not observed. Association between OS in PCNSL patients achieved standard interim 2D CR versus PR was not observed. 3DVR<65% is associated with inferior OS in pts with chemotherapy responsive PCNSL. 3DVR assessment potentially improves identification of poor prognosis PCNSL.

References:

1)Van der Meulen M, Postma AA, Smits M, Bakunina K, Minnema MC, Seute T, et al. Extent of radiological response does not reflect survival in primary central nervous system lymphoma. Neurooncol Adv. 2021;3(1)
2)Ellingson BM, Aftab DT, Schwab GM, Hessel C, Harris RJ, Woodworth DC, et al. Volumetric response quantified using T1 subtraction predicts long-term survival benefit from cabozantinib monotherapy in recurrent glioblastoma. Neuro Oncol. 2018;20(10):1411-8.
3)Abrey LE, Batchelor TT, Ferreri AJ, Gospodarowicz M, Pulczynski EJ, Zucca E, et al. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. J Clin Oncol. 2005;23(22):5034-43.

T cell CD62L expression following nivolumab therapy is associated with long term response to rituximab-nivolumab in treatment naïve follicular lymphoma: results from the 1st FLOR study

Koldej R[1,2], Barraclough A[3], Lee S[4], Morgan H[1,2], Holzwart N[1,2], Koshy M[4], Smith C[4], Chong G[5], Gilbertson M[6], Keane C[7], Ting S[8], Churilov L[9], Ritchie D[1,2,10], Hawkes E[11]
[1]Royal Melbourne Hospital, Melbourne, Australia, [2]University of Melbourne, Melbourne, Australia, [3]Fiona Stanley Hospital, Perth, Australia, [4]Austin Health, Melbourne, Australia, [5]Ballarat Regional Intergrated Cancer Centre, Ballarat, Australia, [6]Monash Health, Melbourne, Australia, [7]University of Queensland, Brisbane, Australia, [8]Eastern Health, Melbourne, Australia, [9]Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, [10]Peter MacCallum Cancer Centre, Melbourne, Australia, [11]Olivia Newton-John Cancer Research and Wellness Centre, Melbourne, Australia

Aim: Inter-tumoral T cell dysfunction and efficacy of immune checkpoint inhibitors (ICI) in follicular lymphoma (FL) has been well described. Few studies have examined the impact of frontline ICI on circulating immunity in FL. We hypothesised that immune dysregulation in peripheral blood would be present in treatment naïve FL and would correlate to response to frontline ICI.

Method: PBMC samples from 34 untreated FL patients receiving rituxibab (R) and nivolumab (nivo) in the 1st FLOR trial (Hawkes JCO 2021) were collected at baseline, after 4 cycles of nivo (PET-CT2) and after 6 months of nivo±R (PET-CT4). Immune profile was assessed using a single-tube 29 antibody FACS panel. Results were correlated with centrally determined PET response (Lugano criteria).

Results: FL patients exhibited increased TRegs and NK cells with dysregulated expression of multiple immune checkpoints on T cells at baseline.

To assess biomarkers of response, patients were stratified into sustained CR (CR achieved by PET-CT4 and maintained for 6 months without evidence of relapse, n=15) vs PR/PD (n=21). Baseline expression of PD-1 on T and NK cells did not correlate with sustained response.

Expression of CD62L was significantly downregulated across T cell subsets at PET-CT2 in PR/PD patients ($P<0.05$) and maintained at baseline levels in those patients who achieved a sustained CR. At this early timepoint final patient responses had not been established with most patients having either progressive or stable disease.

Conclusion: Grade 1-3A treatment naïve FL is associated with significant dysregulation of peripheral blood T and NK cells prior to therapy. Early downregulation of CD62L on T cells of patients treated with nivo was associated with the inability to achieve long term complete responses and may indicate aberrant T cell activation and/or migration to the tumour site which impacts on long term response to therapy.

Long-term efficacy and safety of zanubrutinib in patients with relapsed/refractory marginal zone lymphoma: final analysis of the MAGNOLIA (BGB-3111-214) trial

Opat S[1], Leitch S[2], Browett P[3], Sobieraj-Teague M[4], Walker P[5], Hawkes E[6], Ho S[7], Talaulikar D[8], Tedeschi A[9], Hu B[10], Linton K[11], McKay P[12], Jin J[13], Sun M[14], Zinzani P[15], Ke X[16], Thieblemont C[17], Ardeshna K[18], Bijou F[19], Zhou K[20], Marasca R[21], Mociková H[22], Liang Z[23], Xu J[24], Tankersley C[24], Delarue R[25], Co M[24], Trotman J[26]

[1]Monash Health and Monash University, Clayton, Australia, [2]North Shore Hospital, Auckland, New Zealand, [3]Auckland City Hospital, Grafton, New Zealand, [4]Flinders Medical Centre, Bedford Park, Australia, [5]Peninsula Private Hospital, Frankston, Australia, [6]Box Hill Hospital, Box Hill, Australia, [7]St George Hospital, Kogarah, Australia, [8]Australian National University, Canberra, Australia, [9]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [10]Levine Cancer Institute/Atrium Health, Charlotte, USA, [11]Division of Cancer Sciences, Manchester Cancer Research Centre, Manchester, UK, [12]Beatson West of Scotland Cancer Centre, Glasgow, UK, [13]The First Affiliated Hospital, Zhejiang University, Hangzhou, China, [14]Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China, [15]Institute of Hematology "Seràgnoli," University of Bologna, Bologna, Italy, [16]Peking University Third Hospital, Beijing, China, [17]Université Paris Cité, Assistance publique-Hôpital de Paris, Hôpital Saint-Louis, Hemato-oncology, Paris, France, [18]University College London Hospitals, London, UK, [19]Institut Bergonié, Bordeaux, France, [20]Henan Cancer Hospital, Zhengzhou, China, [21]Department of Medical Sciences, Section of Hematology, University of Modena and Reggio Emilia, Modena, Italy, [22]Department of Clinical Hematology, University Hospital Královské Vinohrady and Third Faculty of Medicine, Charles University, Prague, Czech Republic, [23]BeiGene (Beijing) Co, Ltd, Beijing, China, [24]BeiGene USA, Inc, San Mateo, USA, [25]BeiGene Switzerland GmbH, Basel, Switzerland, [26]Concord Repatriation General Hospital, University of Sydney, Concord, Australia

Aim: MAGNOLIA (NCT03846427) primary results led to approval of zanubrutinib, a next-generation Bruton tyrosine kinase inhibitor, for relapsed/refractory marginal zone lymphoma (MZL); final results are reported.

Method: MAGNOLIA was a phase 2, multicenter, single-arm study in relapsed/refractory MZL treated with ≥1 prior CD20-directed regimen. Patients received zanubrutinib 160 mg twice daily until disease progression or unacceptable toxicity. The primary endpoint was overall response rate (ORR) by independent review committee (IRC) per Lugano criteria. Secondary endpoints included investigator-assessed ORR, duration of response (DOR), progression-free survival (PFS), overall survival (OS), and safety. Efficacy was assessed by positron emission tomography–based Lugano criteria (IRC-confirmed fluorodeoxyglucose-avid disease) or computed tomography–based criteria (non-avid disease).

Results: By May 4, 2022, 68 patients were treated (median age, 70 years). MZL subtypes included extranodal (38.2%), nodal (38.2%), splenic (17.6%), and unknown (5.9%). Most patients (89.7%) received prior chemoimmunotherapy; 32.4% had refractory disease at study entry. Sixty-six patients were evaluable for efficacy (median follow-up, 28.0 months) (**Table**). ORRs were 64.0% (extranodal), 76.0% (nodal), 66.7% (splenic), and 50.0% (unknown subtype); complete response rates were 40.0%, 20.0%, 8.3%, and 25.0%, respectively. Median DOR, PFS, and OS were not reached. At study completion, 31 patients deriving benefit rolled over to a long-term extension study (NCT04170283); 24 discontinued due to disease progression and 5 due to adverse events (AEs), 2 required prohibited medications, and 1 withdrew consent. The most common treatment-emergent AEs were bruising (23.5%) and diarrhea (22.1%). The most common grade ≥3 AEs were neutropenia (8.8%) and COVID-19 pneumonia (5.9%); 5 patients died due to unrelated AEs. Hypertension occurred in 3 patients and atrial fibrillation and flutter in 1 each; none led to treatment withdrawal.

Conclusion: Zanubrutinib continues to be effective with high response rates and durable disease control. Zanubrutinib is generally well tolerated; no new safety signals were observed.

Table. Efficacy Results[a]

	IRC (n=66)		Investigator (n=66)
	PET and/or CT	CT only[b]	PET and/or CT
ORR, n (%) [95% CI]	45 (68.2) [55.6-79.1]	44 (66.7) [54.0-77.8]	50 (75.8) [63.6-85.5]
Complete response, n (%)	17 (25.8)	16 (24.2)	19 (28.8)
Partial response, n (%)	28 (42.4)	28 (42.4)	31 (47.0)
Stable disease, n (%)	13 (19.7)	16 (24.2)	10 (15.2)
Progressive disease, n (%)	6 (9.1)	5 (7.6)	5 (7.6)
24-month DOR rate [95% CI], %	72.9 [54.4-84.9]	66.8 [46.4-81.0]	60.8 [44.8-73.6]
PFS rate at 24 months [95% CI], %	70.9 [57.2-81.0]	64.9 [51.2-75.6]	57.9 [44.8-68.9]
OS rate at 24 months [95% CI], %	85.9 [74.7-92.4]		

CT, computed tomography; PET, positron emission tomography.
[a] n=2 excluded from analysis (centrally confirmed transformation to diffuse large B-cell lymphoma); [b] Sensitivity analysis using only CT-based Lugano criteria regardless of PET status at baseline.

Incidence, prevalence, and mortality of Waldenström macroglobulinemia (WM) in Australia

Talaulikar D[1,2], Gao L[3], Nguyen D[3], Bohingamu Mudiyanselage S[3], Tang B[4], Zhao F[5]
[1]Canberra Health Services, Canberra, Australia, [2]College of Health and Medicine, Australian National University, Canberra, Australia, [3]Deakin Health Economics, Institute for Health Transformation, School of Health & Social Development, Faculty of Health, Deakin University, Melbourne, Australia, [4]BeiGene USA, Inc, San Mateo, USA , [5]BeiGene AUS PTY Ltd, Sydney, Australia

Aim: WM is a rare type of lymphoid neoplasm. There is limited information on its incidence, prevalence, and mortality in Australia. This study aims to examine the epidemiology of WM and predict 30-year trends in incidence and prevalence in Australia.

Method: All WM cases from Jan 2009 to Dec 2018 in Victoria, Tasmania, Australian Capital Territory, and Queensland were extracted from the Australian cancer registry database using the *International Statistical Classification of Diseases* (*ICD-10-AM* code C88.0, histology code 9761). Australian Institute of Health and Welfare–established methods and DisMod II were used to calculate incidence, prevalence, and mortality rates. Thirty-year incidence and prevalence rate predictions were modeled using a least-squares linear regression, and Kaplan-Meier (KM) survival analysis was constructed. All analyses were stratified by sex, age group, and year, when applicable.

Results: The crude annual incidence rate of WM was higher in males than in females (63-116 vs 28-70 per 10,000,000 person-years), with an increased crude incidence trend over 10 years (male: coefficient, 4.17; *P*=.020; female: coefficient, 2.32; *P*=.087). Age-standardized incidence rates ranged from 42 to 78 per 10,000,000 person-years. WM prevalence was lowest in the 50- to 59-year age group (83-207 and 53-89 per 10,000,000 persons for males and females, respectively) and highest in the ≥80-year age group (485-1319 and 159-525 per 10,000,000 persons, respectively). A continuous increase in the prevalence of WM was predicted (male: coefficient, 44.87; *P*<.001; female: coefficient, 31.29; *P*<.001). Females had a lower mortality rate than males (under 5-18 vs 7-41 per 10,000,000 persons). The mortality rate was highest in 2016 and lower in recent years. The KM curve showed that the 10-year survival rate was 42%, regardless of sex.

Conclusion: The incidence and prevalence of WM in Australia have been increasing over the last decade, while mortality and survival have improved since 2016.

A multicentre comparative audit of Hodgkin Lymphoma patients receiving ABVD chemotherapy with or without routine GCSF prophylaxis

De Zylva B[1], Dohrmann S[2], Leung T[1], Ratnasingam S[2]
[1]*Northern Health, Epping, Australia,* [2]*University Hospital Geelong, Geelong, Australia*

Aim: Granulocyte Colony Stimulating Factor (G-CSF) can be used to maintain ABVD (Doxorubicin, Bleomycin, Vinblastine, Dacarbazine) dose intensity in Hodgkin Lymphoma (HL)[1]. Prior studies support intermittent[2,3] or routine[1,4] prophylaxis, but there is no widely accepted standard. This comparative study explores the toxicities and adverse events (AE) in patients receiving and not receiving routine G-CSF, to further guide its use.

Method: Retrospective multicentre audit in two community hospitals on adults with HL receiving ABVD (2017-2022). Demographics, G-CSF use and AE data was obtained from records. Statistical and univariate analyses employed Chi-Square and Fisher Exact testing.

Results: 55 patients were identified; median age 43years. 33(60%) received Universal Prophylaxis(UP) and 22(40%) received no UP. Baseline demographics (*Table1*) revealed an older median age, higher Hasenclever score and more advanced disease in the UP group.

Table1 – Baseline Demographics

	UP n=33 (range) [%]	**No UP n=22 (range) [%]**
Median Age	51 (21-87)	32 (21-75)
Median ECOG	0 (0-2)	0 (0-2)
Median ABVD cycles	6 (2-6)	6 (1-6)
Early Stage Favourable	8 [24]	5 [23]
Early Stage Unfavourable	9 [27]	5 [23]
Advanced Stages	16 [49]	12 [54]
Hasenclever≤2	19 [58]	14 [64]
Hasenclever>2	14 [42]	8 [36]

There was a significantly higher incidence of Grade 4(G4) neutropenia and unplanned admissions with febrile neutropenia (FN) in those without UP (*Table2*), due to omission of G-CSF. 57% of those admitted were age ≥60. G-CSF toxicity was more frequent in the UP group, but this did not result in considerable hospitalisation. Subgroup analysis of patients age ≥60 (n=19) demonstrated a higher incidence of unplanned admissions with FN and one death relating to omission of G-CSF, in those without UP.

Table2 – Universal vs No Universal Prophylaxis Cohort Comparison of AE

	UP n=33 [%]	**No UP** n=22 [%]	**p-value**
Grade 4 Neutropenia (no. of events)	13	31	<0.00001
Delayed Chemotherapy (no. of events)	7	2	0.296
Dose Reduction (no. of patients)	5 [15]	5 [22.7]	0.476
FN admissions	4 [12]	7 [31.8]	0.074
FN requiring ICU/HDU	0	3 [13.6]	0.059
GCSF Omission related unplanned admits	0	7 [31.8]	<0.001
GCSF Omission related deaths	0	1 [4.5]	0.4
GCSF Toxicity (no. of patients)	17 [51.5]	5 [22.7]	0.032
GCSF Toxicity related unplanned admits	0	1 [4.5]	0.4

Conclusion: This study supports the use of universal G-CSF prophylaxis for ABVD in HL, particularly those age ≥60. Significantly more G4 neutropenia and unplanned admissions with FN were seen in those without UP. Prospective larger cohort studies are needed to further support this hypothesis.

References:

- Ho P, Sherman P, Grigg A. Intermittent granulocyte colony-stimulating factor maintains dose intensity after ABVD therapy complicated by neutropenia. European Journal of Haematology 2012; 88; 416-421.
1. Evens AM, Cilley J, Ortiz T, Gounder M, Hou N, Rademaker A et al. G-CSF is not necessary to maintain over 99% dose intensity with ABVD in the treatment of Hodgkin lymphoma: low toxicity and excellent outcomes in a 10-year analysis. British Journal of Haematology. 2007 April 24; 137, 545-552.
2. Cheung MC, Price A, Graczyk J, Buckstein R, Chan KKW. Granulocyte-colony stimulating factor (G-CSF) in secondary prophylaxis for advanced stage Hodgkin Lymphoma treated with ABVD chemotherapy: A cost effectiveness analysis. Blood [Poster]. 2014; 124 (21): 737.
3. Silvestri F, Fanin R, Velisig M, Barillari G, Virgolini L, D Zaja et al. The role of granulocyte colony-stimulating factor (filgrastim) in maintaining dose intensity during conventional-dose chemotherapy with ABVD in Hodgkin's disease. Tumori. 1994 Dec 31; 80(6): 453-458.

A phase 1 study with the novel B-cell lymphoma 2 (Bcl-2) inhibitor BGB-11417 as monotherapy or in combination with zanubrutinib in patients with NHL, or Waldenström macroglobulinemia (WM): preliminary data

Verner E[1,2], Soumerai J[3], Lasica M[4], Opat S[5,6], Cheah C[7,8,9], Chan H[10], González Barca E[11], Tedeschi A[12], Hilger J[13], Fang Y[13], Simpson D[13], Tam C[6,14]
[1]Concord Repatriation General Hospital, Concord, Australia, [2]University of Sydney, Sydney, Australia, [3]Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, USA, [4]St Vincent's Hospital Melbourne, Fitzroy, Australia, [5]Monash Health, Clayton, Australia, [6]Monash University, Clayton, Australia, [7]Department of Haematology, Sir Charles Gairdner Hospital and PathWest Laboratory Medicine, Nedlands, Australia, [8]Medical School, University of Western Australia, Crawley, Australia, [9]Linear Clinical Research, Nedlands, Australia, [10]NorthShore Hospital, Auckland, New Zealand, [11]Institut Català d'Oncologia-Hospitalet, IDIBELL, Universitat de Barcelona, Barcelona, Spain, [12]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [13]BeiGene (Shanghai) Co., Ltd., BeiGene USA, Inc., Shanghai/San Mateo, China/USA, [14]Alfred Hospital, Melbourne, Australia

Aim: BGB-11417-101 (NCT04277637), an ongoing, first-in-human, phase 1/1b dose-escalation/expansion study, assessed BGB-11417 (a highly selective Bcl-2 inhibitor), as monotherapy or in combination with zanubrutinib, a next-generation Bruton tyrosine kinase inhibitor, in NHL (follicular lymphoma, diffuse large B-cell lymphoma [DLBCL], mantle cell lymphoma (MCL), marginal zone lymphoma [MZL]) or WM.

Method: Patients received BGB-11417 (40mg/80mg/160mg/320mg/or 640mg once daily [QD]) with dose ramp-up. In combination cohorts, patients received zanubrutinib (320mg QD or 160mg twice daily) 8-12 weeks before BGB-11417. DLTs were assessed by a Bayesian logistic regression model. Responses were assessed per Lugano criteria.

Results: By 15May2022, 45 patients received BGB-11417: monotherapy (≤640mg; NHL=28, WM=6) or combination (MCL=11; 9 received ≤160mg, 2 were in zanubrutinib pretreatment). No MTD was reached for NHL at doses ≤640mg. Dose-escalation is ongoing for WM (monotherapy) and MCL (combination). Median follow-up was 6.5 months (range, 0.4-25.3; monotherapy) and 4.8 months (range, 0.4-8.9; combination). The most common treatment-emergent AEs were nausea (38%) and fatigue (24%) for monotherapy and contusion and neutropenia (27% each) for combination. The most common grade ≥3 TEAEs were neutropenia (monotherapy=12%; combination=9%) and thrombocytopenia (combination only=9%). Treatment was discontinued by 25 (monotherapy: PD=22; AE=1; other=2) and 2 (combination: PD=2). No tumor lysis syndrome occurred. Of 23 patients with NHL with first response assessments (most below recommended phase 2 dose [RP2D]), 3 responded (DLBCL=2; MZL=1), with 1 CR. With MCL combination treatment, 6 patients (55%) responded. With WM monotherapy, 1 of 4 evaluable patients had a minor response (80mg); 3 of 6 patients had hemoglobin increases (>20 g/L), and all remain on treatment.

Conclusion: Initial data show encouraging safety and antitumor activity of BGB-11417 in NHL and WM. MTD was not reached at doses ≤640 mg QD. Low-grade TEAEs and grade ≥3 neutropenia were manageable. Longer follow-up for BGB-11417 ± zanubrutinib at the RP2D is needed.

Interventions and therapy at end of life in haematological malignancy: A retrospective cohort study on healthcare utilisation and palliative care use

Shaw B[1,2], Parker C[1,2], Shortt J[1,2], Opat S[1,2], Poon P[1,2]

[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia

Aim: Patients with haematological malignancy (HM) have treatment- and disease-related factors that increase rates of hospitalisations and invasive procedures, including close to the end of life. We aim to describe the healthcare utilisation and interventions associated with the intensity of HM therapy prior to death.

Method: Retrospective cohort study of adult patients with HM who were deceased at an Australian non-transplant tertiary hospital network (5 hospital sites, catchment of 1.5 million people) undertaken between 01/10/2019-31/07/2022. Data were extracted from medical records. Descriptive and univariate analysis (chi^2 test) was performed to assess associations between factors.

Results: 229 patients were included (median age 77 years, 65% male, 58% born outside Australia). In the final 30 days of life 65% presented to the ED, 22% had an ICU admission, 22% had an invasive procedure, 48% received disease-modifying therapy, 61% received red cells and 46% received a platelets. 74% were referred to palliative care, median time from referral to death of 13 days and one-third referred within the last 5 days of life.

Participation in a clinical trial within the last 30 days of life was associated with receiving disease-modifying therapy (p=0.03), platelet transfusions (p=0.01) and ICU admissions (p=<0.01). Intensive chemotherapy patients were more likely to be admitted to ICU (p=<0.01), hospitalised for the last 14 or 30 days of life (p=0.01, p=<0.01) and less likely to have a resuscitation limitation (p=<0.01). Those not on therapy were more likely to have a resuscitation limitation (p=<0.01) and not be hospitalised before death (p=<0.01).

Conclusion: Patients with HM had high rates of hospitalisation and therapy use at end of life, with frequent transfusion and critical care admissions. Clinical trial participation and intensive chemotherapy further corresponded with intense healthcare utilisation. Palliative care referral frequently occurred within days of death, limiting the symptom/psychosocial benefits of this multidisciplinary service at end of life.

Table: Interventions, hospitalisation and supportive care by treatment group

	Number	OR	95% CI	P value
Received therapy within 30 days of death				
All patients	111 (48.4%)			
On a clinical trial	24 (64.9%)	2.23	1.07-4.63	0.027
Receiving intensive chemotherapy	36 (64.3%)	2.35	1.26-4.39	0.006
Low dose (non-intensive) therapy	37 (69.8%)	3.19	1.16-6.16	<0.001
Resuscitation limitation on admission				
All patients	123 (53.7%)			
On a clinical trial	15 (40.5%)	0.53	0.26-1.08	0.089
Receiving intensive chemotherapy	20 (35.7%)	0.38	0.20-0.71	0.003
Low dose (non-intensive) therapy	25 (47.2%)	0.71	0.38-1.32	0.290
Active monitoring/no current therapy	45 (77.6%)	4.12	2.08-8.20	<0.001
ICU admission within 30 days of death				
All patients	50 (21.8%)			
On a clinical trial	14 (37.8%)	2.64	1.24-5.62	0.002
Receiving intensive chemotherapy	17 (30.4%)	1.85	0.93-3.67	0.034
Low dose (non-intensive) therapy	10 (18.9%)	0.79	0.36-1.71	0.475
Active monitoring/no current therapy	5 (8.6%)	0.26	0.10-0.70	<0.001
Died in the acute hospital				
All patients	123 (53.7%)			
On a clinical trial	23 (62.2%)	1.51	0.73-3.11	0.276
Receiving intensive chemotherapy	36 (64.3%)	1.78	0.95-3.32	0.076
Low dose (non-intensive) therapy	23 (43.4%)	0.58	0.31-1.08	0.095
Active monitoring/no current therapy	31 (53.4%)	0.99	0.54-1.79	0.964

Clonal and transcriptional determinants of post-CAR T cell relapse in B-cell acute lymphoblastic leukaemia

Kuzich J[1,2,3], Talarmain L[1,2], Vassiliadis D[1,2], Guirguis A[1,2,3], Chan J[1,2], Beavis P[1,2], Darcy P[1,2], Ng A[3,4], Dawson M[1,2,3]

[1]Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia, [3]Clinical Haematology, Peter MacCallum Cancer Centre, Melbourne , Australia, [4]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Aim: The most common pattern of treatment failure following CD19-targeting CAR-T cell therapy is MRD-negative remission followed by relapse. The rare cells that persist despite this strong therapeutic pressure remain entirely uncharacterised. We sought to determine the clonal origin, transcriptional dynamics and targetable vulnerabilities of these “CAR-T tolerant persister cells.”

Method: We developed an immunocompetant mouse model of murine CAR-T cells targeting CD19 on a syngeneic, BCR::ABL1 driven B-ALL, that faithfully recapitulates MRD-negative remission followed by relapse. We applied SPLINTR, a lineage-tracing method utilising expressed molecular barcodes to profile the transcriptional (scRNAseq) and epigenetic (scATACseq) features of single leukaemia clones prior to treatment, in remission, and following relapse.

Results: CAR-T induced significant clonal restriction of relapsing B-ALL. Moreover, the rare clones that comprised relapsed disease were the same across multiple mice and present in only low-frequency in pre-treatment samples and in mice receiving control T cells. This implies that relapse potential is a pre-determined property of rare, specific clones.

While relapse-fated clones were transcriptionally indistinguishable prior to treatment, they underwent substantial, and clone-specific transcriptional adaptation following CAR-T. In contrast to highly concordant adaptive responses *within* individual clones across different recipients, the transcriptional phenotype of the relapsed bulk leukaemia was highly heterogeneous *between* clones, with an absence of identifiable shared resistance mechanisms.

Remarkably however, we identified a rare cell state, shared by each relapse-fated clone, with a distinct, previously uncharacterised, and therapeutically targetable transcriptional program, highly suggestive of a founder state acquired by cells persisting during the MRD-negative period.

Conclusion: The potential for relapse following CAR-T, and the adaptive processes that lead to resistance, are clone intrinsic, heritable properties. Whilst the transcriptional profile of bulk relapsed B-ALL following CAR-T is highly heterogeneous, we have identified that relapse-fated clones share a unique and previously undescribed founder state that may be targetable to prevent relapse after CAR-T.

Molecular MRD assessment is strongly prognostic in patients with NPM1-mutated AML receiving venetoclax-based non-intensive therapy

Othman J[1,2,3,4], Tiong I[5,6,7], O'Nions J[8], Dennis M[9], Mokretar K[10], Ivey A[5], Austin M[11], Latif A[18], Cross J[12], Fong C[7], Galli S[13], Khan A[14], Krishnamurthy P[15], Loo S[6], Pillai S[16], Potter N[3], Taylor T[17], Russell N[2], Wei A[6], Dillon R[2,3]

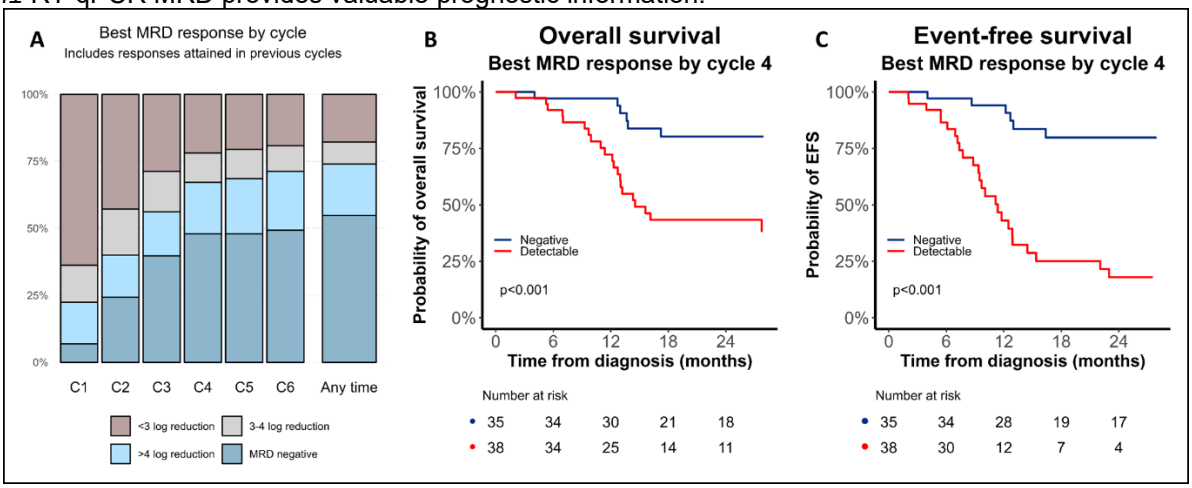
[1]Royal North Shore Hospital, St Leonards, Australia, [2]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [3]Department of Medical and Molecular Genetics, King's College London, London, United Kingdom, [4]University of Sydney, , Australia, [5]The Alfred Hospital, Melbourne, Australia, [6]Peter MacCallum Cancer Centre, Melbourne, Australia, [7]Austin Health and Olivia Newton John Cancer Research Institute, Melbourne, Australia, [8]University College London , London, United Kingdom, [9]The Christie, Manchester, United Kingdom, [10]Cancer Genetics, Synnovis, London, United Kingdom, [11]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, [12]University Hospital Bristol, Bristol, United Kingdom, [13]Frimley Park Hospital, London, United Kingdom, [14]The Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, [15]King's College Hospital, London, United Kingdom, [16]Royal Stoke University Hospital, Stoke-on-Trent, United Kingdom, [17]Nottingham University Hospital, Nottingham , United Kingdom, [18]Queen Elizabeth University Hospital, Glasgow, United Kingdom

Aim: Measurable residual disease (MRD) in patients with *NPM1*-mutated AML treated with intensive chemotherapy is strongly prognostic and can be used to guide therapeutic decisions (Ivey, NEJM 2016; Balsat, JCO 2017), its value in those receiving venetoclax combinations is unknown despite high reported efficacy in this genotype. Flow cytometric MRD is predictive in unselected patients receiving venetoclax and azacitidine but appears less discriminative in the *NPM1*-mutated subgroup (Pratz, JCO 2021). We aimed to assess the prognostic impact of NPM1 RT-qPCR MRD using a large international real-world cohort.

Method: Patients treated with venetoclax and low-dose cytarabine (LDAC) or hypomethylating agents (HMA) were identified from cohorts in the United Kingdom and Australia. Inclusion criteria were i) newly-diagnosed AML with *NPM1* mutation, ii) frontline treatment with venetoclax combination, iii) achievement of CR/CRi and iv) at least one bone marrow MRD assessment in the first 4 cycles of therapy. MRD was performed at reference laboratories using established RT-qPCR assays.

Results: 73 patients meeting the inclusion criteria were identified from 34 hospitals, with median age 72.1 and median follow-up 27 months. Forty patients (55%) achieved MRD negativity as their best response and a further 14 (19%) a reduction of <4 $\log_{10}$ from baseline. Most patients (90%) achieved their deepest response by the end of cycle 4 (Figure 1a). Patients achieving bone marrow MRD negativity by this time had a 2-year overall survival of 80% compared to 44% in those remaining MRD positive (Figure 1 b,c). On multivariable analysis MRD was the strongest prognostic factor. 21 patients electively stopped therapy in MRD negative remission after a median 8 cycles of therapy, with 2-year treatment-free RFS of 88%. Outcomes were similar with HMA and LDAC-based therapy.

Conclusion: In patients with *NPM1*-mutated AML attaining remission with venetoclax combination therapies, *NPM1* RT-qPCR MRD provides valuable prognostic information.



Multiple myeloma-derived circulating extracellular vesicles affect normal human stromal cell behaviour and promote tumor progression: a multi-omic approach

Reale A[1,2], Xu R[1], Carmichael I[1], Khong T[1], Bingham N[1], Fang H[3], Chen M[1], Ramachandran M[1], Greening D[3], Spencer A[1,2]
[1]Alfred Hospital, Melbourne, Australia, [2]Monash University, Melbourne, Australia, [3]Baker Institute, Melbourne, Australia

Multiple myeloma-derived circulating extracellular vesicles.DOCX (could not be inserted)

Next generation sequencing RNA Fusion Panel for the diagnosis of haematological malignancies – an Australian first.

Ngo T[1], Goh A[1], Leong E[1], Fedele P[1,2], Shortt J[1,2], Dorwal P[1], Yeh P[1,2]
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia

Aim: The detection of fusion genes are critically important in the management of haematologic malignancies. We sought to validate a next generation sequencing (NGS) RNA Fusion Panel for the diagnosis of haematological malignancies defined by gene rearrangements, and identify novel RNA fusions that drive haematological malignancies.

Method: 20 patients with haematological malignancies defined by gene rearrangements were identified, including acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML) and myeloid/lymphoid neoplasm with eosinophilia (MLN-Eo). Their diagnostic peripheral blood or bone marrow aspirate RNA samples were sequenced using the TruSightTM RNA Fusion Panel, which is NATA accredited (Table 1). Secondary and tertiary analysis was performed using the Illumina DRAGEN RNA pipeline and PierianDx Clinical Genomics Workspace platform. Validation was performed against traditional methods of detecting gene rearrangements, such as Cytogenetics, FISH and RT-PCR.

Results: Our comprehensive NGS RNA fusion screening tool was able to identify RNA fusion genes in the 20 patients analysed. Examples include ETV6::RUNX1 and KMT2A::AFF1 in ALL (Figure 1), KMT2A::MLLT3 in AML, BCR-ABL in CML and FIP1L1::PDGFRA in MLN-Eo. In one case of eosinophilia, the FIP1L1::PDGFRA gene rearrangement was detected by PCR and our comprehensive RNA fusion panel but not by Cytogenetics/FISH. This represents a unique utility of the RNA-based NGS panel as a diagnostic tool. It is anticipated that the RNA fusion panel will be able to identify novel and rare gene rearrangements to assist with diagnosis, guide treatment decisions and facilitate enrolment into clinical trials. For example, novel fusions in Philadelphia-like B-ALL and myeloproliferative neoplasms could be detected with this powerful diagnostic tool.

Conclusion: Our comprehensive NGS RNA fusion panel was successful in diagnosing haematological malignancies that are driven by gene rearrangements. It also has the potential to identify novel and rare gene rearrangements that may be missed by traditional methods such as Cytogenetics and FISH.

Table 1: Example haematogical genes covered by the TruSight™ RNA Fusion Panel (507 genes in total)							
AFF1	BCR	ETV1-6	FLT3	JAK1-2	MLLT3	NPM1	RARA
ABL1	CBFB	FGFR1-4	GATA1	KMT2A	MYC	PDGFRA/B	RUNX1
BCL2, BCL6	CEBPA	FIP1L1	IRF4	MECOM	MYH11	PML	TP53

Figure 1: Detection of KMT2A-AFF1 rearrangement in a patient with B-ALL using the TruSight™ RNA Fusion Panel

Tisagenlecleucel for relapsed/refractory high grade B cell lymphoma: a single centre Australian experience

Wayte R[1], Vanguru V[1,2], Brown C[1,2], Abadir E[1,2], Bryant C[1,2], Dunkley S[1], Khoo L[1], McCulloch D[1,2], Burge C[1], Malau P[1], Dubosz K[1], Rasko J[3,4], Larsen S[1,2], Ho P[1,2]
[1]Department of Haematology, Royal Prince Alfred Hospital, Camperdown, Australia, [2]University of Sydney, Camperdown, Australia, [3]Department of Cell and Molecular Therapies, Royal Prince Alfred Hospital, Camperdown, Australia, [4]Gene and Stem Cell Therapy Program, Centenary Institute, University of Sydney, Camperdown, Australia

Aim: Tisagenlecleucel (tisa-cel), a commercial CD19 directed chimeric antigen receptor T-cell immunotherapy, has been available in New South Wales (NSW) for relapsed/refractory diffuse large B cell lymphoma (DLBCL) since 2020. We report real world outcomes with tisa-cel for DLBCL at a single centre from September 2020 to December 2022.

Method: Outcome data from 50 consecutive patients with DLBCL successfully infused with tisa-cel at Royal Prince Alfred Hospital in NSW from September 2020 to December 2022 were collected prospectively. Cytokine release syndrome (CRS) and immune effector-cell associated neurotoxicity syndrome (ICANS) were graded as per ASTCT guidelines. Response was assessed by PET (Deauville criteria) at one, three, six and twelve months post infusion with clinical review thereafter. Outcome data was censored on 31 March 2023.

Results: 50 patients received tisa-cel in the specified time period with 82% receiving bridging therapy. CRS occurred in 82% of patients, with 78% grade 1 or 2. ICANS occurred in 10% of patients with no events grade 3 or higher. Median follow up was 12 months (range 1-30 months). The overall response rate (ORR) was 50% with a median progression free survival (PFS) of 16 months. 46% of patients achieved a complete response. The median overall survival (OS) was not reached. Patients with normal LDH at time of infusion had more favourable outcomes than those with high LDH, with hazard ratio of progression 2.49 (95% CI 1.03-6, p=0.01).

Conclusion: PFS and OS at our centre were higher than the published JULIET (long term) study (PFS 16 months compared to 2.9 months) with an improved safety profile (1). This difference is likely due to a high proportion (66%) of patients having disease control with normal LDH at time of infusion. This suggests disease control at time of infusion is a key indicator of response.

1. Schuster SJ, Tam CS, Borchmann P, Worel N, McGuirk JP, Holte H, et al. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol. 2021;22(10):1403-15.

Total body irradiation-based myeloablative T-cell-replete haploidentical stem cell transplantation: a single institution experience

Tan S[1], Mineely S[1], O'Flaherty E[1], Ritchie D[1,2], Bajel A[1,2]
[1]Royal Melbourne Hospital, Parkville, Australia, [2]Peter MacCallum Cancer Centre, Parkville, Australia

Aim: Allogeneic stem cell transplant (alloSCT) is a curative option for some haematological malignancies, however optimal conditioning intensity and modality prior to haplo-identical alloSCT for high-risk lymphoid malignancies remain uncertain. We report our experience with T-cell-replete haplo-identical alloSCT with myeloablative total body irradiation (TBI)-based conditioning.

Method: Adult patients with high-risk lymphoid malignancies who received Fludarabine (30mg/m2 on D-7 to D-5) and TBI-12Gy (1.5Gy twice daily D-4 to D-1) conditioning and post-transplant Cyclophosphamide (50mg/kg on D+3 and D+4), Tacrolimus and Mycophenolate with a haploidentical peripheral blood stem cell graft between August 2019 and February 2023 were identified from our database.

Results: Eleven patients were included in this analysis **(Table 1).** Median age was 39 years (20-55). Nine (73%) patients were in negative measurable-residual-disease (MRD) using multiparameter flow cytometry (FC) pre-transplant. Disease status at transplant included first complete remission (CR1) in 4 patients (FC MRD negative = 4) and CR2 in 7 patients (FC MRD negative = 5). Three out of four patients with intermediate disease risk index (DRI) have high-risk cytogenetic abnormalities. Two patients required donor specific antibody (DSA) reduction pre-conditioning.

All patients engrafted with a median time to neutrophil and platelet recovery of 19 (17-26) and 24 (29-44) days, respectively. All patients achieved complete donor chimerism by Day+100 post-transplant. Five patients (46%) developed cytokine release syndrome (max grade 1). With a median follow-up of 13 months (4 – 47), the 1-year overall (OS) and relapse-free survival was 100%. One patient each was diagnosed with grade II acute graft-versus-host disease (GVHD) and mild chronic GVHD. Two patients (18%) had moderate chronic GVHD. The cumulative incidence of graft and relapse free survival (GRFS) at 1 year was 72%.

Conclusion: T-cell-replete haploidentical-SCT using myeloablative TBI-based conditioning is a safe and feasible option resulting in high rate of OS and GRFS post-transplant.

Table 1: Baseline characteristics of all patients (N=11)

Median Age, **y (range)**	39 (20-55)
Diagnoses, **no. (%)**	
T-lymphoblastic leukaemia/lymphoma	4 (36)
B-acute lymphoblastic leukaemia	3 (27)
Mixed phenotype acute leukaemia	2 (18)
Follicular lymphoma	1 (9)
Chronic myeloid leukaemia with lymphoid blast crisis	1 (9)
Comorbidity Index (HCT-CI), **no. (%)**	
0-2	6 (54)
3+	5 (46)
Disease risk index (DRI), **no. (%)**	
Intermediate	4 (36)
High	5 (46)
Very High	2 (18)
Pre-transplant MRD (flow or PET), no. (%)	
Positive	2 (18)
Negative	9 (73)

The addition of ATG to reduced-intensity conditioning transplantation from a matched unrelated donor results in similar GRFS but increased TRM when compared to T cell replete matched sibling donor transplantation

Koo R[1,2,3,4], Wong E[1,2,3,4,5], Koldej R[3,4], Ritchie D[1,2,3,4]
[1]Bone Marrow Transplant Service, Royal Melbourne Hospital, , Melbourne, Australia, [2]Clinical Haematology Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia, [3]Australian Cancer Research Foundation Translational Research Laboratory, Royal Melbourne Hospital, Melbourne, Australia, [4]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, , Melbourne, Australia, [5]Clinical Haematology, Austin Health, , Heidelberg, Australia

Aim: Anti-thymocyte globulin (ATG) reduces graft-versus-host disease (GVHD) in reduced-intensity conditioning allogeneic stem cell transplantation (RIC-alloSCT) but the impact on relapse varies. Differences in RIC regimens, ATG formulation and dosing may contribute to this variation. We report the impact of ATG on outcomes in patients with acute myeloid leukaemia (AML) and myelodysplasia (MDS) treated with fludarabine and melphalan (FluMel) conditioning.

Method: Between 2016 and 2022, all AML/MDS patients undertaking RIC-alloSCT in our institution with FluMel from a fully HLA-matched sibling donor (MSD) or unrelated donor (MUD) were identified. When indicated, ATG (Thymoglobulin® 4.5mg/kg, in divided doses, day -3 to -1) was utilised. Demographic information, disease characteristics, GVHD incidence and survival were analysed.

Results: Of the 117 patients identified, 61% (n=72) received ATG (100% MUD recipients). All non-ATG recipients obtained grafts from a MSD. Patient and disease characteristics were similar. At 100 and 180 days, the cumulative incidence (CI) of grade 2-4 acute GVHD was reduced in the FluMelATG group (11% vs 27%, *P*=0.04; and 18% vs 48%, *P*=0.002). The CI of chronic GVHD (1-year and 2-years) were similar. ATG did not impact the 2-year CI of relapse (25% vs 22%, *P*=0.7), overall survival (63% vs 68%, *P*=0.3) and GVHD, relapse free survival (GRFS, 37% vs 22%, *P*=0.2). Day 100 non-relapse mortality (NRM) was worse (17% vs 2%, *P*=0.01) in ATG recipients, with sepsis (n=8) as the leading cause of NRM in FluMelATG. EBV reactivation requiring treatment (24% vs 2%, *P*=0.002) and primary graft failure (9% vs 0%, *P*=0.04) were more common in ATG recipients. Median engraftment time was similar.

Conclusion: In this retrospective study of FluMel RIC-alloSCT, MUD recipients receiving ATG had post-transplant outcomes similar to that of MSD recipients. Early NRM was increased.

Quantifying treatment burden of cytomegalovirus reactivation following allogeneic haematopoietic stem cell transplantation.

Ranjit Anderson N[1], Uluitoga S[1], Butler J[1], Curley C[1], Henden A[1,3], Morton J[1], Subramoniapillai E[1], Tey S[1,3], Kennedy G[1,2], Scott A[1,2]

[1]Royal Brisbane and Women's Hospital , Herston, Australia, [2]University of Queensland, St Lucia, Australia, [3]Translational Cancer Immunotherapy, QIMR Berghofer Medical Research Institute, Herston, Australia

Aim: In an era where novel agents are not yet routinely available, we sought to determine risk factors relevant to design of a new pharmacological prophylactic strategy for cytomegalovirus (CMV) reactivation following allogeneic haematopoietic stem cell transplantation (HSCT).

Method: We conducted a retrospective chart review of all patients who underwent allogeneic HSCT between January 2020 to December 2021 at our centre. Patients with clinically significant CMV is defined as those requiring ≥6 days of anti-CMV therapy. Events of interest following treatment included transfusion, hospital readmission and non-CMV infection. Analysis included descriptive statistics, t-test and one-way ANOVA.

Results: There were 220 alloHSCT patients during this period, all T-replete. 62 (28%), required CMV treatment; two with CMV disease. Conditioning/GVHD prophylaxis protocol was the only factor associated with subsequent CMV reactivation (p=0.044), with highest incidence (83%) among CMV-seropositive recipients of reduced-intensity (RIC) or nonmyeloablative (NMA) conditioning with post-transplant cyclophosphamide (PTCy) GVHD prophylaxis. CMV treatment duration also differed significantly by conditioning/prophylaxis (p=0.01), with RIC/NMA PTCy recipients in particular requiring two weeks more anti-viral treatment than other patients (median 41 *vs.* 27 days, p=0.01). Compared to other patients, RIC/NMA PTCy recipients also required significantly more transfusions (packed red cell and platelets) within 60 days of CMV reactivation (3 vs 7units, p=0.01), but did not experience significantly more days in hospital (median 12 *vs.* 13 days, p=0.76) or ≥grade 3 infections (0.7 *vs.* 0.7, p=0.38). One patient experienced treatment failure, but there were no directly related CMV deaths. Overall survival (OS) for CMV-treated patients was significantly inferior to those not requiring treatment (median OS not reached, p=0.03); however, although non-relapse mortality was 35% in RIC/NMA PTCy recipients, this was not significantly different (p=0.35).

Conclusion: We found CMV reactivation occurred predominantly in RIC/NMA PTCy recipients, was associated with longer treatment duration and more transfusions. This patient subgroup appears most in need of novel CMV prophylaxis and treatments.

Use of haplo-identical donor stem cells increase the risk of CMV reactivation in patients receiving post-transplant cyclophosphamide for GVHD prophylaxis

Schwarer J[1], Tan J[1], Muirhead J[1], Cirone B[1], Inam S[1], Curtis D[1]
[1]*Alfred Hospital, Melbourne, Australia*

Aim: Post-transplant cyclophosphamide (PTCy) for GVHD prophylaxis following allogeneic stem cell transplant (alloSCT) increases the risk of CMV re-activation. The aim of this study was to compare the rates of CMV-reactivation in patients receiving PTCy according to donor stem cell source.

Method: A retrospective analysis of the Alfred alloSCT database identified 119 patients receiving PTCy for GVHD prophylaxis between February 2015 and December 2022. Valganciclovir prophylaxis was used for all patients where the donor or recipient were CMV IgG positive. CMV PCR was performed weekly for 100 days post-transplant and beyond 100 days when indicated. CMV reactivation was defined as any positive CMV PCR within 6-months of alloSCT.

Results: Overall, transplant characteristics for high risk of CMV reactivation (age, conditioning, acute GVHD) were similar (Table). Patients transplanted with haplo-identical donor stem cells were 3-fold more likely to develop CMV viraemia > 1,000 IU/mL that required treatment. The median maximum CMV viral load was significantly higher in those receiving haplo-identical cells with CMV viraemia occurring earlier post-transplant in this donor group as well (Figure). CMV disease was uncommon irrespective of the donor stem cell source. AML was an independent risk factor for CMV viraemia >1000IU/mL on multivariate analysis (p=0.004)

Table: Characteristics of the 119 patients according to stem cell source.

Donor stem cell source	MSD (n=36)	MUD (n=42)	Haplo (n=41)	p-value
Median Age, y	58	62	52	0.13
Conditioning MAC, n (%)	5 (14)	3 (7)	11 (27)	0.05
Acute GVHD grade II-IV, n (%)	4 (22)	9 (31)	7 (19)	0.51
CMV pos. serology, n (%)	32 (89)	30 (71)	31 (78)	0.17
CMV reactivation, n (%)	21 (66)	18 (60)	24 (77)	0.34
CMV > 1000 IU/mL, n (%)	4 (12)	3 (10)	10 (32)	0.05
CMV treatment, n (%)	6 (19)	5 (17)	15 (48)	0.01
CMV disease, n (%)	3 (9)	0 (0)	3 (10)	0.24

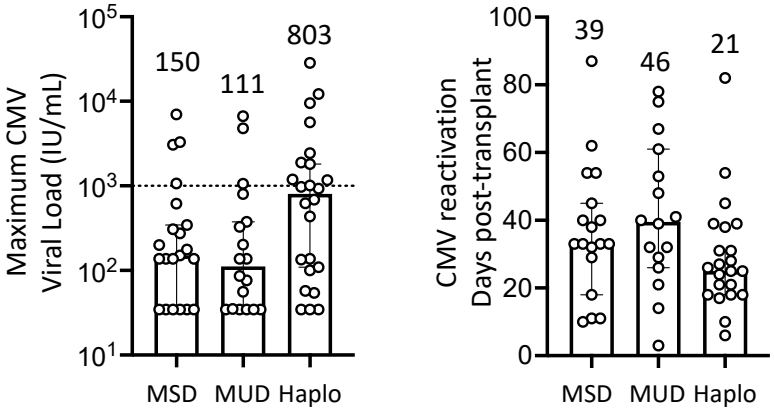


Figure: Maximal viral load and time post-transplant for matched sibling (MSD), matched unrelated (MUD) and haplo-identical donor transplants. Median levels are shown.

Conclusion: Haplo-identical donor source has a higher risk of clinically significant CMV reactivation despite routine prophylaxis. These results suggest consideration of alternate CMV prophylaxis strategies that may be more effective.

Allogeneic stem cell transplantation achieves long-term remissions in patients with mantle cell lymphoma, including TP53-mutated disease

Lew T[1,2,3], Cliff E[1,4], Dickinson M[1,5], Tam C[1,3,6], Seymour J[1,3], Blombery P[1,3,7], Bajel A[1,5], Ritchie D[1,3,5], Khot A[1,5]

[1]Peter Maccallum Cancer Centre And Royal Melbourne Hospital, Melbourne, Australia, [2]Blood Cells and Blood Cancer Division, Walter and Eliza Hall Institute of Medical Research,, Melbourne, Australia, [3]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, Australia, [4]Program on Regulation, Therapeutics and Law, Brigham and Women's Hospital, Harvard Medical School, Boston, Australia, [5]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia, [6]Alfred Hospital and Monash University, Melbourne, Australia, [7]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia

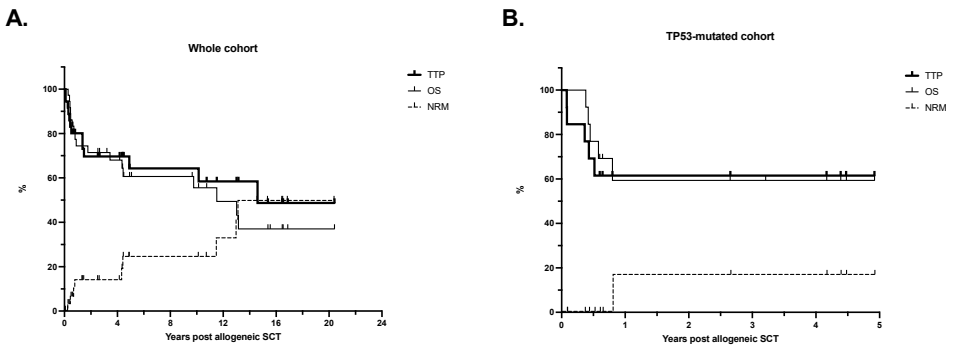
Although chemoimmunotherapy and autologous stem cell transplant (autoSCT) is effective for the majority of patients (pts) with mantle cell lymphoma (MCL), those with *TP53*-mutated disease have a dismal prognosis.

Aim: To describe the clinico-pathologic characteristics and outcomes of a cohort of pts treated with allogeneic SCT (alloSCT) for MCL.

Method: We retrospectively reviewed 36 consecutive pts undergoing alloSCT for MCL at the Royal Melbourne Hospital and Peter MacCallum Cancer Centre from July 2000 to Feb 2023. Baseline clinico-pathologic variables, transplant and outcome data were collected. The Kaplan-Meier method was used to estimate progression-free survival (PFS), time-to-progression (TTP), non-relapse mortality (NRM) and overall survival (OS). *TP53* mutations were evaluated by next-generation sequencing.

Results: Twenty-two (61%) pts underwent alloSCT in first response and 14 (39%) for relapsed disease. Median age was 52.5 (range 33-71) years. Thirteen pts had disease with confirmed *TP53* mutations; 11 proceeded to alloSCT in first response and two after disease relapse. The remaining pts did not have molecular testing. Induction regimens were predominantly cytarabine-containing (n=27; 75%). Most pts received reduced-intensity conditioning (n=29; 81%). The donor source was sibling in 47% of cases, matched unrelated in 39%, with four haploidentical donors (11%) and one cord. With a median follow-up of 10.8 years for the whole cohort, the estimated 10-year PFS and OS rates were 48% (95%CI 30-64%) and 56% (95%CI 36-72%), respectively. 2-year NRM was 14% (95%CI 5-33%). At a median follow-up of 4.2 years for pts with *TP53*-mutated MCL, the 4-year PFS and OS rates were 51% (95%CI 21-75%) and 59% (95%CI 28-81%), respectively, with one instance of NRM.

Conclusion: AlloSCT is potentially curative for ~50% of pts with MCL, including those with *TP53*-mutated disease. In this high-risk subset, early alloSCT represents a viable alternative to conventional approaches which have been demonstrated to be ineffective in prospective studies.



TTP	36	18	11	8	4
OS	36	20	13	8	4
NRM	36	18	11	8	4

TTP	13	5	5	4	4
OS	13	6	6	5	4
NRM	13	5	5	4	4

Allogeneic haematopoietic stem cell transplant patients in the intensive care unit – a ten year retrospective cohort study

Heydon C[1], Perera T[1]
[1]*Te Whatu Ora, Wellington, New Zealand*

Background/Aim: Patients undergoing haematopoietic stem cell transplants (HSCT) who are admitted to an intensive care unit (ICU) have a high mortality. Some studies show an almost universal mortality for ventilated patients with a second organ failure, whilst later studies, including one from our unit, identified significant protective factors against mortality, including admission to ICU pre-engraftment. This study replicates an earlier study from our unit, looking at mortality statistics for a 10 year cohort of alloHSCT recipients admitted to ICU and compares them to the prior cohort and published data from other centres. It also looks at admission trends to ICU and outcome predictors.

Methods: A ten-year retrospective audit was performed, looking at all alloHSCT patients who were admitted to Wellington Hospital ICU between January 2012 and December 2021. Primary outcomes were ICU and in-hospital mortality and survival at 6 and 12 months post ICU admission. A comparison with an identical cohort from 2002-2012 has been performed, and univariate and multivariate analyses are also to be performed.

Results: 232 patients underwent alloHSCT for the given period. There were 111 admissions to ICU representing 75 patients. ICU mortality occurred in 50% of patients, while in-hospital mortality occurred in 57%. Six and twelve month mortality was 76% and 79% respectively. Comparison with the prior cohort demonstrated similar rates of admission and mortality. Univariate and multivariate analyses are ongoing.

Conclusion: Survival rates post-ICU admission are consistent with those reported internationally, but interestingly there has been little change over the last ten years, despite ongoing advances in transplant conditioning, supportive care and ICU care. This may be due to transplants being performed on more complex patients over the last ten years, and it will be interesting to see if this is reflected in differences in the disease risk index (DRI) and comorbidity scores, analysis of which is still pending.

Intensive Pegcetacoplan Dosing in the Management of Acute Haemolysis as Part of the 307 Open-Label Extension Study

Szer J[1], Griffin M[2], Kelly R[2], Deeren D[3], Goh Y[4], Tse E[5], Usuki K[6], Wong R[7], Horneff R[8], Yan L[8], Yeh M[9], Savage J[9]
[1]Peter MaCallum Cancer Centre, Melbourne, Australia, [2]St. James University Hospital, Leeds, UK, [3]Algemeen Ziekenhuis (AZ) Delta, Roeselare, Belgium, [4]Singapore General Hospital, , Singapore, [5]Queen Mary Hospital, University of Hong Kong, , Hong Kong, [6]NTT Medical Center Tokyo, Tokyo, Japan, [7]Sir YK Pao Centre for Cancer & Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, , Hong Kong, [8]Swedish Orphan Biovitrum AB, Stockholm, Sweden, [9]Apellis Pharmaceuticals, Waltham, USA

Aim: To assess the effects of intensive subcutaneous (SC) or intravenous (IV) pegcetacoplan in patients with paroxysmal nocturnal haemoglobinuria (PNH) who are experiencing an acute haemolysis (AH) event.

Method: Patients included in the analysis were enrolled in the ongoing 307 study (NCT03531255) of pegcetacoplan in PNH patients who completed Phase 1–3 trials (PHAROAH, PEGASUS, PALOMINO, PADDOCK, PRINCE). Patients with AH (lactate dehydrogenase [LDH] >2x ULN and 1 new or worsening sign or symptom of haemolysis) warranting acute intervention were eligible for intensive pegcetacoplan SC (1080 mg every 24 hours for 3 doses) or IV dosing (1 dose of 1080 mg) at the investigator's discretion. Haemoglobin and LDH levels during the AH event are described. Safety: adverse event (AE) incidence and severity.

Results: 13 of 137 patients in study 307 received intensive pegcetacoplan treatment for AH and all showed evidence of response (ie, LDH decrease) within the first week, with 69% reaching LDH <2x ULN at day 14–19. Haemoglobin improved for all patients. Four of 13 patients required a transfusion to treat AH. LDH decreased between days 1 and 2 in 8 of 12 evaluable patients (50% of intensive SC- and 100% IV-treated patients) and all 13 patients at days 7–12.

From day 1 - 21 of intensive dosing, 54% of patients experienced treatment-emergent AEs (TEAEs); 3 (23%) experienced serious AEs (1 event of sepsis possibly related to study drug, 2 events of haemolysis not related to study drug). 77% of TEAEs were mild or moderate. No AEs of meningitis or thrombosis occurred. No AEs led to treatment discontinuation. There were no deaths.

Conclusion: These data support effective AH event management with intensive SC or IV pegcetacoplan dosing and show that LDH levels can be rapidly controlled and haemoglobin levels stabilised. Intensive pegcetacoplan treatment was safe and well tolerated.

Characteristics of paediatric and adult patients with atypical haemolytic uraemic syndrome (aHUS) treated with eculizumab – an Australian clinical audit.

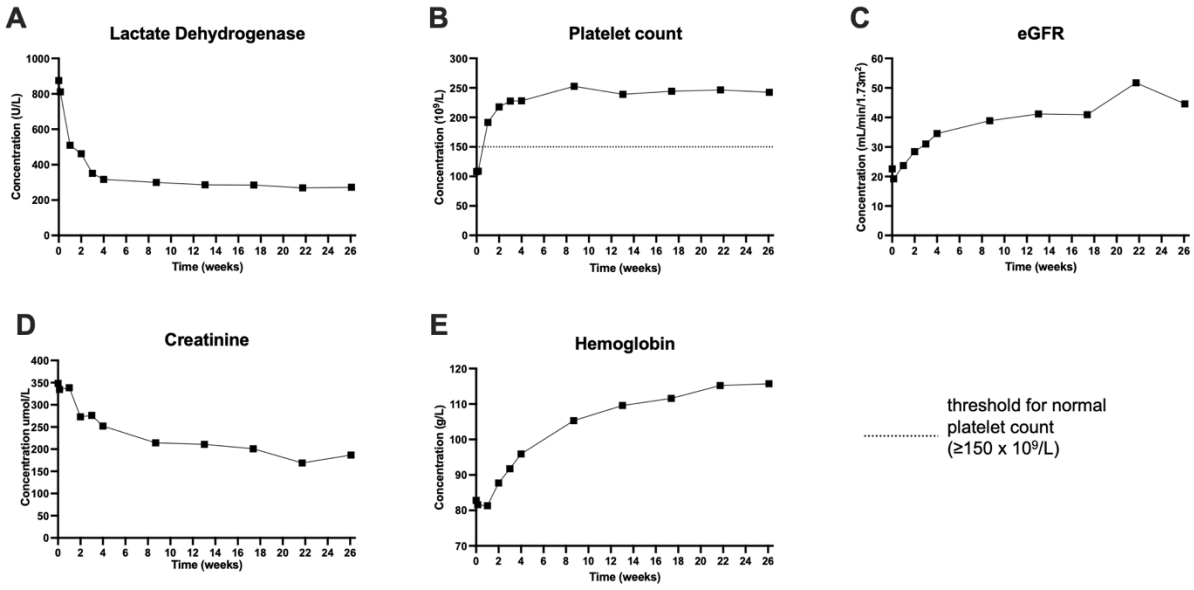
Hsu D[1], Kurtkoti J[2], Raniga J[3], Tan S[4], Ryan J[5], Leadbetter J[6], Kausman J[7], Stootman L[6], Isbel N[8]
[1]Haematology Department, Liverpool Hospital, Liverpool, Australia, [2]Gold Coast University Hospital, Southport, Australia, [3]Alexion Pharmaceuticals Australasia, Macquarie Park, Australia, [4]Royal Melbourne Hospital, Parkville, Australia, [5]Monash Medical Centre, Clayton, Australia, [6]WriteSource Medical, Lane Cove, Australia, [7]Royal Children's Hospital, Parkville, Australia, [8]Princess Alexandra Hospital, Woolloongabba, Australia

Aim: This study addresses a gap in real-world data on the demographic and clinical characteristics of patients with aHUS treated with eculizumab in routine clinical practice.

Method: This observational cohort study involved retrospective collection of medical record data for patients from six Australian hospitals who were prescribed Pharmaceutical Benefits Scheme (PBS)-funded eculizumab for aHUS between 1-Dec-2014 and 31-Dec-2021. Statistical analyses were descriptive. Overall survival was estimated using Kaplan-Meier methods. Subgroup analyses of patients who had no recorded eculizumab use prior to this study (treatment-naïve) are presented here.

Results: There were 55 treatment-naïve patients (72% of patients). 38 (69%) patients were female. Median (min, max) age was 41 years (3, 82 years). Patients accessed PBS-subsidised eculizumab using the modified French TMA score (31%) or through an ADAMTS13 result (69%). Diagnosis occurred median (min, max) 4 days (-2, 60 days) after hospitalisation. 23 (42%) patients experienced ≥1 ICU admissions. 23 (42%) patients were at high risk of aHUS relapse/recurrence. 27 (49%) patients had genetic testing, with ≥1 pathogenic/possibly pathogenic variant identified in 9 patients. 30 (55%) patients developed kidney failure before first eculizumab use, defined as dialysis within 30 days before eculizumab treatment and an eGFR of ≤15ml/min in the last measurement before eculizumab treatment. Extrarenal manifestations were primarily cardiovascular (39 patients; 70%). The most common precipitating event for aHUS was infection (12 patients; 22%). 26 (47%) patients received plasma exchange within 30 days before first eculizumab use. Eculizumab treatment conferred an improvement in laboratory parameters (Figure 1). The survival rate was 92.1% at 12 months.

Conclusion: This study describes the characteristics of aHUS patients in a real-world setting and provides evidence for eculizumab as an effective therapy for aHFigure 1: Change in laboratory parameters after initiation of eculizumab in treatment-naïve patients.



Tmprss6 siRNA treatment in mice stops hepcidin reduction across pregnancy

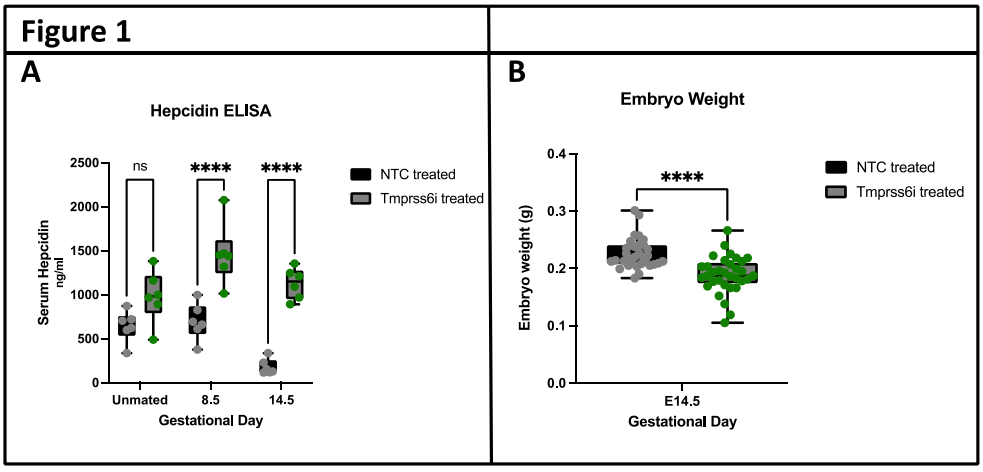
Fielding K[1], Ataide R, Bennett C, Pettikiriarachchi A, Clucas D, Schaeper U, Pasricha S
[1]*WEHI, Melbourne, Australia,* [2]*Austin Health, Melbourne, Australia*

Aim: Hepcidin levels are suppressed during human and murine pregnancy. It is unknown whether hepcidin levels are primarily the result of iron deficiency/utilisation or whether there is a pregnancy-specific mechanism for hepcidin suppression. We reasoned that *Tmprss6* inhibition would cause constitutive BMP-SMAD signalling and help determine the mechanism of antenatal hepcidin suppression.

Method: Female wildtype C57BL/6 mice were treated every 21 days with a GalNAc-siRNA conjugate targeting *Tmprss6* (Silence Therapeutics GmbH, Berlin, Germany) or a non-targeting control (NTC) siRNA conjugate. Mice were mated and monitored for pregnancy. Samples were collected from pregnant mice humanely euthanised at E8.5 or E14.5.

Results: Treatment with *Tmprss6* siRNA inhibited hepatic *Tmprss6* mRNA expression (unmated fold change 0.077, p<0.0001).
qPCR *Hamp* expression was significantly increased in *Tmprss6* siRNA-treated mice, compared to NTC-treated mice, at all timepoints. Serum hepcidin, by ELISA, was higher in *Tmprss6* siRNA-treated mice than in NTC-treated mice at E14.5 (1126ng/ml vs 178.5, p<0.0001). In unmated mice, serum hepcidin was not significantly elevated (640.9 vs 980.1ng/ml, p=0.224).
Across NTC treated pregnancy, *Hamp* expression and serum hepcidin reduced at E14.5 compared to unmated (*Hamp* fold change E14.5 0.293, p=0.0367; serum hepcidin E14.5 178.5 vs 640.9, p=0.0247). In contrast, *Tmprss6* siRNA treatment caused elevated hepcidin through pregnancy (E14.5 1126 vs unmated 980ng/ml, p=0.99); Fig 1a.
Maternal liver iron was unchanged between groups. *Tmprss6* siRNA reduced placental iron, and fetal weight (0.189 vs 0.226g, p<0.0001), Fig 1b. At E8.5/E14.5, *Tmprss6* siRNA reduced Mean Cell Volume (E14.5: 41.42 vs 51.72fL, p<0.0001) and Mean Cell Haemoglobin (E14.5: 11.20 vs 15.68pg, p<0.0001); at E14.5 only, maternal anaemia was seen with *Tmprss6* siRNA (haemoglobin 9.4 vs 13.85g/L, p=0.0004).

Conclusion: Disruption of *Tmprss6* increased hepcidin and ablated the hepcidin fall over pregnancy. This may suggest that any pregnancy-related factor which directly suppresses hepcidin cannot overcome *Tmprss6* knockdown. *Tmprss6* siRNA treatment resulted in iron-restricted erythropoiesis, reduced placental iron and impacted fetal development, despite unchanged liver iron stores.



Genotype to phenotype: what are those dots?

Perchard M[1]
[1]*The Royal Children's Hospital, Flemington, Australia*

Aim: To present two cases of a rare, interesting genetic disorder with abnormal neutrophil granulation that may represent a characteristic phenotype not previously described in literature.

Background: We present two cases of abnormal neutrophil granulation with an associated genetic mutation or deletion in the WDR81 gene. This genetic condition is associated with neurodevelopmental abnormalities including lissencephaly, developmental delay, seizure disorders and cerebellar ataxia. The role of WDR81 protein not clearly understood. It is one of the Beige and Chediak Higashi (BEACH) domain proteins with hypothesised roles in transport and migration of proteins. In cell culture it has been found that WDR81 knock out cells had enlarged intracellular vesicles which stained for lysosomal markers.

Discussion: Two patients with known WDR81 gene mutations were identified to have abnormal granules in neutrophils on blood film examination. The patients had no evidence of infection or inflammation at the time of the blood tests. The granules are large, purple staining and generally evenly distributed in the cytoplasm. There were no Dohle bodies or other features of infection/inflammation. The patients did not have a diagnosis of mucopolysaccharide storage disorder or Chediak-Higashi syndrome. Neither patient had a history of recurrent infections or sepsis with no evidence of immunodeficiency. Whole genome sequencing demonstrated mutations or deletions in the WDR81 gene on chromosome 17 location 17p13.3 for both patients. A literature review was conducted to further understand the role of WDR81.

Conclusion: WDR81 mutations are associated with neurodevelopmental disorders that have typical structural brain abnormalities, seizures and developmental delay. The characteristic neutrophil inclusions may be related to hypothesised transport function of the WDR81 protein resulting in the neutrophil inclusions seen on our patients. Further studies are required to assess the function of these abnormal lysozymes and to determine if this is part of the WDR81 syndrome spectrum.

A novel telemedicine therapeutic intervention to improve the health of post-transplant patients in their home

Ma D[1], Fennessy K[1], Kliman D[3]
[1]St Vincent's Hospital Sydney, Darlinghurst, Australia, [2]School of Clinical Medicine, St Vincent's Healthcare Clinical Campus, Faculty of Medicine and Health, UNSW Sydney, , Kingsington, Australia, [3]Royal North Shore Hospital, St Leonards, Australia

Aim: Telecommunication technologies have been used to deliver medical consultation and healthcare services (telemedicine) remotely for >2 decades. The feasibility of providing safe and cost-effective supervision of chemotherapy remotely to rural communities in Australia was reported in 2012 (1). Lower survival and reduced physical and mental well-beings are well-recognized late effects of haematopoietic stem cell transplant (HCT). Supervised exercise and mindfulness-based stress management (MBSM) trainings have shown promise, mainly in the inpatient setting. The aim of this single centre trial is to evaluate the feasibility of a 6-week, virtual-based programme of combined exercise and MBSM to post-HCT patients at home.

Method: Adult patients >6 months post allogeneic HCT in our post-HCT clinic were invited to participate. 21 of 24 consented participants attended an in-person session, followed by six once-weekly exercise and MBSM trainings via videoconferencing. Objective and subjective assessments that have been shown to reflect functional outcomes were performed via the Internet at pre-training, and 3-, 6- and 12-months post-intervention and evaluated using a linear mixed effects model.

Results: The median age of the participants was 53 (median time post-HCT 37 months). Compared to pre-intervention, six-minute walk and sit-to-stand tests improved significantly at 3 months (both $p<0.01$) and 12 months ($p<0.01$ and <0.05 respectively). Dominant handgrip strength increased at 3, and 12 months ($p<0.01$). Increased FACT-BMT total and FACT-G scores were found at 3 months ($p<0.01$). 80% of participants rated the telemedicine intervention programme highly and no adverse events were reported.

Conclusion: This novel 6-week virtual-based exercise and MBSM program was feasible and resulted in potentially sustained improvement of physical and mental well-beings of HCT survivors. Virtual based healthcare service is highly relevant particularly during pandemics. To our knowledge, this world-first study has the longest follow-up for internet based combined modality training program reported to date and randomised control trial is under way.

References:
(1) Sabesan S et al Internal Medicine Journal 2012 (https://doi.org/10.1111/j.1445-5994.2011.02537.x)

Outcomes of patients with haematological malignancy admitted to ICU and factors associated with survival: a retrospective cohort study

Shaw B[1,2], Kearns M[1], Shortt J[1,2], Murfin B[1]
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia

Aim: Historically the mortality rate of critically unwell patients with haematological malignancy (HM) has been high. However, with the availability of prognostically disruptive targeted therapies, long-term outcomes for HM are becoming more favourable, increasing the impetus to provide ICU support. We aimed to describe the outcomes of patients with HM admitted to ICU and the factors associated with poorer survival to inform future practice.

Method: A retrospective cohort study of patients admitted under Haematology to ICUs at an Australian tertiary hospital network (servicing a population of 1.5M people) between 2012-2022. Data were extracted from medical records with descriptive statistics reported and univariate analysis (chi^2 test) performed for associations.

Results: 360 patients were included, median age 68yrs (21 to 92yrs) with 32.2% female. 47% of patients were aged ≥70yrs and 16% had a treatment limitation documented. The majority patients were admitted to ICU with sepsis (Table 1). ICU mortality rate was 19.7% and hospital admission mortality rate 30%. Approximately half of patients survived for >6 months after ICU admission (Figure 1). The median APACHE-III score in the cohort was 84 (IQR=67-101) with higher median APACHE-III scores in those dying in ICU (median 110 [IQR=85-122]) and those who died in the same hospital admission (median 102 [IQR 84-117]). Death in ICU and death during the hospital admission were associated with invasive ventilation (p=<0.01), requiring dialysis (p=0.02,0.03), albumin ≤15 (p=<0.01), bilirubin >30 (p=<0.01) and creatinine >100 (p=<0.01) compared to all patients with HM admitted to ICU. Age ≥70y, inotrope use or temp ≥38.0^{o}C were not associated with dying in ICU.

Conclusion: Mortality rates were similar to other studies, including Australian[1-4] . Risk of dying in ICU is likely associated with higher APACHE-III scores and poor underlying organ function but not necessarily with age. Further research is needed to identify subsets of patients most benefitting from ICU.

Table 1: Categories of acute illness leading to ICU admission

	Number of pts	**% of total**
Infection, sepsis, pneumonia	171	55.2%
Haem disorder, thrombocytopenia, neutropenia	43	13.9%
Cardiac, AMI, cardiac arrest, cardiogenic shock	28	9.0%
Respiratory, COPD, hypoxia	25	8.1%
GI bleeding, neoplasm, perforation, obstruction	13	4.2%
Neurological, ICH, stroke, CNS infection, seizure	13	4.2%
Other metabolic	7	2.6%
Renal disorders	7	2.6%
Orthopaedic	3	1.0%

Figure 1: Rates of survival over time from ICU admission date

References : **1**. Parakh S et al Intern Med J. 2014 Nov. **2.** Alp E et al. Aust Crit Care 2018 Nov. **3.** Hampshire P et al. Crit Care 2009. **4.** Asdahl PH et al. Intensive Care Med. 2020 Apr.

Economic evaluation of prophylactic immunoglobulin versus prophylactic antibiotics in haematological malignancies: preliminary results from the RATIONAL feasibility trial

Carrillo de Albornoz S[1,2], Higgins A[1], Petrie D[2], Irving A[1,2], Fanning L[2], Wood E[1], van Tonder T[1], Wellard C[1], Reynolds J[1,19], Weinkove R[3,4,5], Crispin P[6], Dendle C[7,8], Gilbertson M[9], Johnston A[10,11], Keegan A[12], Pepperell D[13], Trotman J[14,15], Pullon H[16], Weston H[17], Morrisey O[18,19], McQuilten Z[1,9]

[1]School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia, [2]Centre for Health Economics, Monash University, Melbourne, Australia, [3]Te Rerenga Ora Wellington Blood & Cancer Centre, Wellington, New Zealand, [4]Cancer Immunotherapy Programme, Malaghan Institute of Medical Research, Wellington, New Zealand, [5]Department of Pathology & Molecular Medicine, University of Otago Wellington, Wellington, New Zealand, [6]Canberra Hospital, Canberra, Australia, [7]Monash Infectious Diseases, Monash Health, Melbourne, Australia, [8]School of Clinical Sciences, Monash University, Melbourne, Australia, [9]Haematology, Monash Health, Melbourne, Australia, [10]Royal Hobart Hospital , Hobart, Australia, [11]University of Tasmania, Hobart, Australia, [12]Australian Red Cross Lifeblood, , Australia, [13]Department of Haematology, Fiona Stanley Hospital, Perth, Australia, [14]Concord Repatriation General Hospital, Sydney, Australia, [15]University of Sydney, Sydney, Australia, [16]Department of Haematology, Waikato Hospital, Hamilton, New Zealand , [17]Sunshine Coast University Hospital, Sunshine Coast, Australia, [18]Department of Infectious Diseases, Alfred Health, Melbourne, Australia, [19]Central Clinical School, Monash University, Melbourne, Australia

Aim: To estimate the 12-month cost-effectiveness of prophylactic immunoglobulin (Ig) versus prophylactic antibiotics in patients with acquired hypogammaglobulinemia (HGG) secondary to haematological malignancies in the RATIONAL trial (ACTRN12616001723471p).

Method: The RATIONAL trial randomised 63 adults with chronic lymphocytic leukaemia, multiple myeloma, or lymphoma to prophylactic Ig or prophylactic antibiotics. Two analyses were conducted:

- Cost-utility analysis (CUA) to assess the incremental cost per quality-adjusted life-year (QALY) gained, using HRQoL data collected with the EuroQol 5-dimension 5-level (EQ-5D-5L) instrument;

1. Cost-effectiveness analysis (CEA) to assess the incremental cost per major infection prevented (Grade ≥3) and per infection (any grade) prevented.

Results: Over the 12-month trial period, the total cost per patient was higher in the Ig group than in the prophylactic antibiotic group (mean difference: AUS$29,140 (95%CI $15,941, $42,340). There were non-significant differences in health outcomes between treatment groups. Patients treated with Ig had fewer QALYs than those given antibiotics (mean difference: -0.072 [95%CI -0.167, 0.023]), more overall infections (mean difference: 0.76 [95%CI -0.33, 1.86]), but fewer major infections (mean difference: -0.26 [95% CI -0.74, 0.21]). The incremental cost-effectiveness ratio (ICER) in the CUA indicated Ig was dominated by antibiotics (i.e., Ig was more costly and associated with fewer QALYs). In the CEA, Ig was dominated by antibiotic treatment when all infections were included (i.e., Ig was more costly and was associated with more infections), and the ICER was AUS$111,262 per major infection prevented.

Conclusion: These preliminary results indicate that, on average and for this group of patients with haematological malignancies and HGG recruited to the RATIONAL feasibility trial, Ig prophylactic treatment may not be cost-effective compared to prophylactic antibiotics. As the RATIONAL trial was not powered to detect infection and QALY differences, further research is needed to confirm these findings in a larger population and over a longer time period.

Understanding transfusion practices and comorbidities in hospitalised elderly patients

Roxby D[1], Sobieraj-Teague M[2], Sinha R[1]
[1]Flinders University, Adelaide, Australia, [2]Flinders Medical Centre, Bedford Park, Australia

Aim: With a higher prevalence of anaemia and high incidence of comorbidities in patients aged 65 years or more, a retrospective study was undertaken to examine the transfusion rates in this patient population

Method: All consecutive separations of patients aged ≥ 65-year-old in a one year period (July 2018 and June 2019) from a tertiary care hospital using the hospital's morbidity dataset were identified. Demographic data collected included: ICD 10 codes (red cell transfusion and comorbidities) and Speciality Related Groups (SRG). Co-morbidities included diabetes, hypertension, chronic kidney disease, chronic pulmonary disease, neurological disorders, heart insufficiency, and malignancy.

Results: A total of 37055 admissions were identified, of which 1732 (4.7%) received at least one unit of red cells. The top 5 admissions receiving a red cell transfusion constituted 47% of the total transfused admissions and belonged to Intensive Care admissions (54.3%), haematology (47.7%), gastroenterology (34.1%), cardiac surgery (21.4%) and colorectal surgery 19.6%) SRGs. Admissions with three or more comorbidities had a higher rate of transfusion compared to admissions with two or less comorbidities, 189/1324 (14%) vs 1543/35731 (4.3%), p<0.001. Comparing the transfused and non-transfused admissions and any comorbidities, the median, interquartile length of stay (days) for admissions with a single comorbidity was 6 (IQR 3-13) vs 1 (1-3), p <0.001, with two comorbidities was 11 (IQR 6-19) vs 4 (2-9), p <0.001], with three comorbidities was 12 (IQR 7-21) vs 6 (3-12), p <0.001, and for more than three comorbidities was 18 (IQR 11-30) vs 8 (4-14), p <0.001.

Conclusion: Patients with increasing numbers of comorbidities received more red cell transfusions compared with those with fewer comorbidities and had longer hospital stays. These initial data will help in directing a larger study to predict the required resources including transfusion and length of stay in elderly patients.

ABO-mismatched platelet transfusions, immune platelet refractoriness, and platelet support

Han M[1,2], Badami K[2]
[1]Christchurch Hospital, Christchurch, New Zealand, [2]New Zealand Blood Service, Christchurch, New Zealand

Aim: Major ABO-incompatible platelet transfusions may result in poor platelet increments – a kind of immune refractoriness. Recently it was shown that ABO-identical, and non-identical platelets can, respectively, prevent or predispose to immune refractoriness[1]. We aimed to study the influence of prior ABO-matched or -mismatched platelet transfusions on immune platelet refractoriness and subsequent platelet support in New Zealand (NZ).

Method: Retrospective analysis of adults and children from five major NZ hospitals who were positive for anti-HLA/HPA between 01 January 2017 to 31 December 2021 were included (*n* = 136). We examined patient demographics, transfusion history including ABO group of prior platelet transfusions, and subsequent transfusions of HLA-matched platelets. Data were obtained from the NZ Blood Service electronic database. Continuous variables are presented as median and range. Frequencies are presented as percentages.

Results: 136 patients tested positive for anti-HLA/HPA. Of these patients, 71 (52.2%) received at least one ABO-mismatched platelet unit prior to testing ('ABO-m patients'), while 65 (47.8%) received only ABO-identical platelets ('ABO-I patients'). 66.2% of ABO-i and 63.4% of ABO-m patients were female.

122 patients (89.7%) required subsequent HLA-matched platelets. ABO-m patients needed more units of HLA-matched platelets (median 17 units, range 2 – 139 units) than ABO-i patients (median 10 units, range 1 – 87 units). ABO-m patients also needed HLA-matched platelet support for longer (median 3.6 months, range 0.02 – 67.1 months) than ABO-i patients (median 1.8 months, range 0 – 32.5 months).

Conclusion: Our small dataset appears not to show a difference between ABO-i and ABO-m patients with respect to testing positive for anti-HLA/-HPA, but it does show that ABO-i patients needed significantly less platelet support than ABO-m patients. While awaiting more definitive results, using ABO-identical platelets when possible may be a good thing from this perspective.

References:

- Cardillo A, Heal JM, Henrichs K, et al. Reducing the Need for HLA-Matched Platelet Transfusion. *N Engl J Med*. 2021;384(25):2451-2452. doi:10.1056/NEJMc2034764

Storage of platelets for transfusion influences cross-talk with malignant HepG2 cells

Hueneburg T[1,2,3], Ward C[2,3], van der Wal D[1], Marks D[1,2]
[1]Australian Red Cross Lifeblood R&D, Sydney (Alexandria), Australia, [2]University of Sydney, Camperdown , Australia, [3]Kolling Institute, St Leonards , Australia

Aim: Approximately 60% of oncology patients receive prophylactic platelet transfusions to prevent bleeding due to low platelet counts following chemotherapy. Platelet-cancer cell cross-talk can lead to platelet activation and detrimental changes in malignant cells, making them more metastatic. Interactions between malignant cells and ex-vivo stored platelets for transfusion have not been investigated. Therefore, this study examined interactions between stored apheresis platelets and malignant cells using a hepatocellular carcinoma (HepG2) cell line.

Method: Apheresis platelets were stored under standard conditions. Samples were taken on day 1, 5, and 7 post-collection. Interactions between platelets and HepG2 cells ± 1.25 µM calcium chloride ($CaCl_2$) were monitored by light transmission aggregometry (n=5). In separate experiments, platelets (n=4) were incubated with adhered HepG2 cells in 24-well plates (37°C, 10 minutes) ± 1.25 µM $CaCl_2$ and ± 2.5 mM Gly-Pro-Arg-Pro (GPRP) to prevent clotting, followed by platelet phenotypic characterisation using flow cytometry. Data were analysed using one and two-way ANOVA; $p<0.05$ was considered significant.

Results: Platelets and HepG2 cells aggregated in the presence of $CaCl_2$. However, the lag-time increased with platelet storage duration ($p=0.0002$). In plate assays, platelet activation measured by phosphatidylserine externalisation ($p<0.0001$) and PAC-1 binding to activated GPIIb/IIIa ($p<0.0001$) was increased following incubation with HepG2 cells/$CaCl_2$ on day 1. In contrast, platelet receptors GPVI, CD41/CD61 and GPIbα decreased upon incubation with HepG2/$CaCl_2$. In general, the degree of receptor reduction was less marked with longer platelet storage. GPRP inhibited the changes in AnnV and platelet receptors, except for CD62P and PAC-1, which were increased in the presence of GPRP ($p<0.0001$ for both).

Conclusion: Platelets for transfusion aggregated and underwent phenotypic changes in the presence of malignant HepG2 cells, which became less marked during *ex vivo* platelet storage. These findings suggest platelet storage may reduce platelet-cancer cross-talk, warranting further investigation.

Evaluating the quality of X-ray irradiated deglycerolised red cells stored in SAGMan

Winter K[1], Webb R[1], Mazur E[1], Dennington P[1], von Wielligh K[1], Marks D[1]
[1]*Australian Red Cross Lifeblood, Alexandria, Australia*

Aim: Rare phenotype red blood cells are routinely cryopreserved and stored at -80°C for over 10 years. Upon thawing and deglycerolisation, red cells are resuspended in saline-adenosine-glucose-mannitol (SAGM) with a limited 24-hour shelf-life. There is a lack of consensus between countries as to whether irradiation of deglycerolised red cells is necessary for patients at risk of transfusion-associated graft-versus-host disease. Our facility has recently introduced X-ray irradiation for blood components, and therefore the quality of X-ray irradiated deglycerolised red cells resuspended in SAGM was assessed.

Method: Matched red cell components (n=21 pairs) were glycerolised with 40% glycerol, frozen at -80°C, deglycerolised using an ACP-215 cell washer and resuspended in SAGM. One of each pair was X-ray irradiated post deglycerolisation, whilst the other remained untreated (control). Red cells were sampled pre-freeze and immediately, 24-, 48- and 72-hours post-irradiation. Red cells were tested for quality indicators including red cell indices, metabolic and biochemical parameters. Irradiated and control red cells were compared using repeated measures ANOVA; $p<0.05$ was considered significant.

Results: There were no significant differences between X-ray irradiated and control groups for volume, haemoglobin (Hb), haematocrit, supernatant Hb immediately post-thaw ($p=0.6425$, $p=0.2314$, $p=0.0609$ and $p=0.3951$ respectively). Haemolysis was higher in X-ray irradiated deglycerolised red cells (0.35±0.14%) than controls (0.32±0.05%) after 24 hours of storage, and rapidly increased to 0.52±0.12% and 0.45±0.09% respectively after 72 hours post-irradiation ($p=0.0772$). Potassium (K^+) release was significantly higher in the irradiated red cells compared to controls at 24-hours post-thaw (6.26±0.95 vs 3.21±1.01 mmol/unit respectively; $p<0.0001$) and was 1.7-fold higher by 72-hours post-irradiation.

Conclusion: X-ray irradiation had a significant effect on K^+ release from deglycerolised red cells during storage. Whilst K^+, and to a lesser extent haemolysis, increased during storage following X-irradiation, the levels at 24 hours (current expiry) were acceptable, as were the other quality parameters.

Ultrarapid Iron Polymaltose infusions are safe for management of iron deficiency

Banakh I[1], Turek M, Niewodowski D, Sriamareswaran R, Yeaman F, Vo L, Churchill T

[1]*Monash Health, Clayton, Australia*

Aim: Iron deficiency is a common condition, especially among patients with kidney and heart failure and inflammatory bowel disease. Intravenous iron is the preferred method of treatment in these patients, but it usually requires prolonged iron polymaltose infusions or multiple administrations of alternative preparations. The aim of the study was to confirm the safety and patient acceptance of ultrarapid iron polymaltose infusions as an alternative to slower treatments and ferric carboxymaltose

Method: An open label, phase 4 study was conducted at a major hospital, with consenting participants diagnosed with iron deficiency and requiring iron polymaltose up to 1,500 mg receiving the infusion over 15 min. The acute adverse event (AE) rates and their severities were compared to historical controls of 1- and 4-h iron polymaltose infusions from a retrospective study of 648 patients from the same study site. Delayed AEs as well as participant infusion acceptability were also studied. Chi squared tests were used to compare rates of adverse reaction between groups on SPSS 24.

Results: Three hundred participants over a 2-year period received ultrarapid infusions of iron polymaltose with an acute AE rate of 18.7% and severe AE rate of 1.0%. The total and mild infusion AE rates were higher compared to those of slower infusions ($p < 0.001$), but comparable for moderate and severe AEs. Delayed reactions occurred in 12.5% of participants, with over 95% of them preferring repeat ultrarapid infusions if required again.

Conclusion: Iron polymaltose can be safely infused at ultrarapid rates when compared to slower infusions, with similar safety to ferric carboxymaltose, offering greater convenience for patients and reduced healthcare costs.

Whole blood for trauma resuscitation: Is it pro-inflammatory?

Tan J[1,2], Aung H[1], Marks D[1,2]
[1]Australian Red Cross Lifeblood, Alexandria, Australia, [2]The University of Sydney, Camperdown, Australia

Aim: Transfusion of one unit of whole blood (WB) product provides a balanced ratio of red cells, platelets and plasma. Recent clinical studies indicate there are limited adverse reactions following transfusion of cold-stored WB. At Lifeblood, a manufacturing method for cold-stored WB is in development, with characterisation of red cells, platelets and plasma proteins. However, there is limited data describing inflammatory mediators in WB products. The aim of this study was to characterise changes in inflammatory mediators in cold-stored WB throughout storage.

Method: WB (n=12) was collected into CPD anticoagulant, held overnight, processed through a platelet-sparing filter, and stored at 2-6°C for 42 days. Samples were taken on day 1, 4, 7, 14, 21, 28, 35 and 42, and platelet-poor-plasma was prepared by centrifugation. Targets were measured by ELISA, cytometric bead array and flow cytometry. Data were analysed using one-way ANOVA comparing each time-point to day 1, with a *post hoc* two-sided Dunnett's t-test.

Results: Following filtration, 99.98% of leukocytes were removed, with 85% platelet recovery. There was a significant increase in soluble platelet-derived factors including PF4 ($p<0.0001$) and sCD62P ($p<0.0001$), and to a lesser extent, sCD40L ($p=0.037$) during storage. The inflammatory mediators HMGB1 ($p=0.748$), S100A12 (EN-RAGE; $p=0.274$) and C5a ($p=0.988$) remained stable throughout storage, whereas C3a increased significantly from day 14 ($p<0.0001$). There was a significant increase in the chemokines RANTES ($p<0.0001$) and MCP-1 ($p<0.001$). IL-6, IL-8, IL-13, MIP-1α and IFN-□ were below the limit of detection. Platelet- and red cell-derived microparticles increased during storage (both $p<0.0001$), whereas the number of white cell-derived microparticles remained low and did not change ($p=0.100$).

Conclusion: This study demonstrates that biological mediators accumulate in cold-stored WB during storage. High concentrations of inflammatory mediators in WB may have clinical consequences for transfusion recipients in a trauma setting, although this is yet to be elucidated.

This work was supported by the ANZSBT Research Fund 2021.

Japanese Encephalitis Virus Antibody Seroprevalence Among Blood Donors Following an Outbreak of Infections in Southern and Eastern Parts of Australia

Hirani R[1,2], Winkler N[3], Baker J[4], Hueston L[5,6], Koirala A[3], O'Sullivan M[5], Hoad V[7], Irving D[1,8], Gosbell I[1,9], Macartney K[3]

[1]Australian Red Cross Lifeblood, Sydney, Australia, [2]Macquarie University, Sydney, Australia, [3]National Centre for Immunisation Research and Surveillance, Sydney, Australia, [4]University of Sydney, Sydney, Australia, [5]NSW Health Pathology Institute of Clinical Pathology and Medical Research, Sydney, Australia, [6]Menzies Institute of Health, Brisbane, Australia, [7]Australian Red Cross Lifeblood, Perth, Australia, [8]University of Technology Sydney, Sydney, Australia, [9]Western Sydney University, Sydney, Australia

Background: Japanese encephalitis (JE) is endemic to Asia and is caused by the mosquito-borne JE virus (JEV) with ~68,000 cases per year. In February 2022, an outbreak of JEV emerged in the southern and eastern parts of Australia. For most people, JEV infection results in mild symptoms or is asymptomatic but 1:250 infections can result in severe outcomes. JEV vaccination is encouraged for individuals with high risk but supplies are limited. With JEV detection in more populated parts of Australia, a human serosurveillance program was conducted.

Methods: Serum samples from all blood donors who attended 9 blood collection centres in areas of high-risk for infection (based on proximity to infected piggeries), and 3 low-risk centres, across 4 states and territories were collected. Samples were sent to the Institute of Clinical Pathology and Medical Research at Westmead Hospital, Sydney for analysis using a JEV-specific total antibody in a defined epitope blocking assay. Any positive samples were further tested for exposure to other local endemic flaviviruses including, Murray Valley encephalitis and Kunjin viruses.

Results: Between 8 August - 17 September 2022, a total of 5,257 serum samples were collected. Preliminary results indicated that from 5018 sample 84 were positive for JEV once exclusions were applied for participants born in a JEV-endemic country or prior vaccination. Overall, there was 2.1% (95% CI 1.6–2.6) seropositivity in areas of high-risk for JEV infection, compared to 1.0% (95% CI 0.6–1.6) in low risk areas.

Conclusions: JEV seroprevalence may be marginally higher among blood donors who live in higher risk JEV areas. However, there was limited data on JEV vaccination status and previous potential exposure can only be extrapolated using country of birth as a proxy. These data could inform targeted allocation of limited JEV vaccines and confirms previous modelling of a small transfusion transmission risk.

Characterisation of caspase activation in fresh, cryopreserved, and apoptotic platelets

Waters L[1], Marks D[1,2], Johnson L[1]
[1]*Australian Red Cross Lifeblood, Alexandria, Australia,* [2]*University of Sydney, Camperdown, Australia*

Aim: Certain characteristics of cryopreserved platelets are aligned with features of apoptosis, including phosphatidylserine externalisation and depolarisation of the mitochondrial membrane. However, many apoptotic features overlap with procoagulant platelets, including caspase activation. The aim of this study was to investigate caspase activation in apoptotic, fresh and cryopreserved platelets.

Methods: Apheresis platelets (day 1 post-collection) were tested fresh, cryopreserved or stimulated with 30 μM of ABT-737 for 2-4 hours at 37°C to generate apoptotic platelets. Fresh platelets were cryopreserved at -80°C with 5-6% dimethylsulfoxide and resuspended in a unit of plasma upon thawing. Caspase 9 and caspase 3/7 activation were assessed by flow cytometry (n=8) and western blotting with densitometry (n=3). Data were analysed using a one-way ANOVA, with $p<0.05$ being considered statistically significant.

Results: Cryopreserved platelets demonstrated significant phosphatidylserine externalisation (69±5% annexin V-positive; $p<0.0001$), and depolarisation of mitochondrial membranes, as demonstrated by a reduction in tetramethylrhodamine (TMRE) sequestration ($p<0.0001$). A high proportion of apoptotic platelets demonstrated activation of caspase 9 and 3/7. Caspase 9 activation was increased in cryopreserved platelets compared to fresh, while caspase 3/7 activation remained comparable. By western blotting, cleaved caspase 3 and 7 were highly abundant in apoptotic platelets, but barely detectable in fresh and cryopreserved platelets.

	Fresh	Cryopreserved	Apoptotic
Caspase 9 (FITC-LEHD-FMK; % positive)	2±1*	27±7*†	69±18
Caspase 3/7 (FITC-DEVD-FMK; % positive)	3±2*	6±4*	69±16
Cleaved caspase 3 (19kDa band)#	1.0±0.4*	1.0±0.2*	309±56
Cleaved caspase 7 (20kDa band)#	1.0±1.6	0.5±0.9	585.4±154.8

*p<0.05 compared to apoptotic
†p<0.05 compared to fresh
#relative to total protein staining and normalised to fresh

Conclusion: These data suggest that a low proportion of fresh and cryopreserved platelets are apoptotic, aligning with our previous findings. Greater clarification of the phenotype of cryopreserved platelets could be discerned by investigating caspase activation in procoagulant and stored platelets.

The impact of JK-null variants on red blood cell biology.

Chong F[1], Dizon P[1,2], Lopez G[1], Millard G[3], Powley T[3], Hyland C[1], Kolarich D[2], **Griffiths R[1]**
[1]R&D, Australian Red Cross Lifeblood, Kelvin Grove, Australia, [2]Griffith University, Southport, Australia, [3]Red Cell Reference Laboratory, Australian Red Cross Lifeblood, Kelvin Grove, Australia

Aim: Kidd (Jk) blood group antigens are present on the red blood cell (RBC) membrane urea transporter (UT-B) glycoprotein. RBCs with the Jk null phenotype lack all antigens but limited studies have explored whether insertion of the UT-B protein occurs in the red cell membrane.

Our aim was to characterise the system wide biological impact of a JK null variant on the donor derived mature RBC proteome of blood donors and developing erythroid cells, using our ex vivo model of erythropoiesis.

Method: RBCs were isolated from routine EDTA blood samples from control and Jk(a-b-) donors and subject to flow cytometry and quantitative proteomics analyses to quantitate the expression of RBC surface proteins.

To characterise RBC protein expression during erythropoiesis, haematopoietic stem cells were differentiated to RBCs using an established erythropoiesis model. The expression of the UT-B protein and other erythroid specific proteins were examined throughout erythroid differentiation in control and Jk(a-b-) cells by flow cytometry and confocal microscopy.

Results: Flow cytometry and proteomics confirmed the absence of UT-B on the surface of mature Jk(a-b) RBCs. Confocal microscopy showed that UT-B production occurred in developing Jk(a-b) erythroid cells but was not integrated into the RBC membrane.

Quantitative proteomics identified that proteins involved in solute transport and membrane-cytoskeleton stabilisation were significantly down-regulated in mature Jk (a-b) RBCs.

Conclusion: We show that the UT-B protein is produced in Jk(a-b-) null erythroid cells but fails to integrate into the RBC membrane. Furthermore, our data suggests that the absence of a functional UT-B on the RBC surface resulted in a down-regulation of other integral RBC membrane proteins. The mechanism and potential clinical consequences of these changes require further investigation.

Impact of Newly Emerging Dengue Outbreak on Blood Supply at a Hospital-based Blood Bank in Oman

Al-Riyami A[1,2], Al-Omairi I[1,2], Al-Hosni S[1,2], Al-Hinai S[1,2], Al-Marhoobi A[2]
[1]Department of Hematology, Sultan Qaboos University Hospital, Muscat, Oman, [2]Department of Hematology, Sultan Qaboos University, Muscat, Oman

Aim: The rapid expansion of dengue fever is an evolving worldwide public health threat. In Oman, all cases of dengue reported in the past were travel-related. However, a short outbreak of indigenous dengue occurred in December 2018. In 2022, the health authorities detected the re-emergence of Dengue (1), leading to recommendations of excluding blood donors from the outbreak areas. This study aims to assess the impact of the newly emerging dengue outbreak on blood supply in a hospital-based blood bank with donation and transfusion facilities.

Method: A retrospective review of records was performed (January - December 2022). The review included number of blood drives, donations, and deferrals. Descriptive statistics were used.

Results: Total number of blood drives in 2022 doubled, increasing from 23 in 2021 to 50 in 2022. However, the total number of voluntary blood donations in 2022 decreased to 6,995 compared to 8,088 donors in 2021, representing a 13.5% drop. This downward trend persisted throughout the year, and was most severe during spring and summer holidays. The number of deferred donors increased to 1,016 in 2022, a 54% rise compared to 464 in 2021. The percent of donor deferrals in blood drives doubled, reaching 43.4% compared to 25% in 2021.

Conclusion: This study confirmed that dengue outbreaks have a significant impact on the blood supply at our hospital-based blood bank. These findings underscore the importance of proactive measures to manage blood inventory during dengue outbreaks, and the development of strategies to overcome the challenges faced by blood bank operations.

References:

- Al Awaidy, Salah T., et al. "Epidemiological and Clinical Characteristics of Patients with Dengue Fever in a Recent Outbreak in Oman: A Single Center Retrospective-cohort Study." Oman Medical Journal 37.6 (2022): e452.

Management of moderate/severe iron deficiency anaemia in stable patients presenting to the Emergency Department: A retrospective audit

Shaw B[1,2], McLeod R[1], Egerton-Warburton D[1], Vilcassim S[1,2], Rushford K[1], Chebrolu P[1], Rosler R[1], Mo A[1,2]
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia

Aim: Guidelines recommend that haemodynamically stable patients presenting with iron deficiency anaemia (IDA) are managed with iron replacement, rather than red cell (RBC) transfusion. We aimed to evaluate the current management of stable patients with moderate/severe IDA in the Emergency Department (ED) to inform future practice.

Method: Following ethics approval, a retrospective cohort study of patients presenting 1/1/2020 to 31/7/2021 at an Australian tertiary hospital network (catchment of 1.5 million people) was conducted. Non-pregnant patients aged≥18 years with moderate/severe IDA (haemoglobin ≤90g/L, ferritin<30 g/L) who presented to, and were discharged from, the network's 3 EDs were identified. Laboratory/clinical data were derived from hospital medical records.

Results: 78 patients included (82% female, median age 43 years). 66 patients (85%) were referred by their GP. 12 patients (15%) had symptoms of IDA. Three patients (4%) received RBC transfusion in ED (median Hb 64g/L, range 51-72g/L); none were haemodynamically unstable. 75 patients (96%) were not transfused (median Hb 79g/L, range 59-89g/L). 72% of patients were given an iron infusion (69% iron carboxymaltose 1g): 36 (46%) in ED and 20 (26%) did not receive iron infusion in ED but were referred to Hospital In The Home (HITH). The proportions infused in ED compared with HITH varied across the sites (table 1). Of those not receiving RBC/iron in ED, 48% were referred to HITH for iron infusion and 28% discharged on oral iron. Median time from HITH referral to iron infusion was 7 days (IQR 5-10d). Re-presentation rate to ED was similar for those given/not given IV iron in ED (13.9%vs19.0%,p=0.53).

Conclusion: Stable patients with moderate/severe IDA can be managed with iron supplementation without RBC transfusion. Many patients were referred by their GP to ED for iron infusion which is available through HITH. This audit was strengthened the referral patterns between GPs and HITH to reduce unnecessary presentations to ED.

Table: Comparison across all 3 emergency department locations

	All patients	Site 1	Site 2	Site 3
N	78	38	19	21
Age (years, median, (IQR))	43 (19-93)	47 (23-93)	42 (20-78)	39 (19-90)
Female (%)	82.1%	78.9%	78.9%	90.5%
% female aged <50 years	69.2%	34.2%	42.1%	76.2%
Presented within business hours	73.1%	81.6%	84.2%	47.6%
Referred by General Practitioner	84.6%	89.5%	78.9%	81.0%
Symptomatic at presentation	15.4%	15.8%	15.8%	14.3%
Transfused RBC	3 pts (3.8%)	1 pt (2.6%)	1 pt (5.3%)	1 pt (4.8%)
Given iron infusion in ED (n,%)	36 (46.2%)	27 (71.1%)	2 (10.5%)	7 (33.3%)
Referred to HITH for iron infusion n(%)	20 (25.6%)	2 (5.3%)	11 (57.9%)	7 (33.3%)
Time to HITH iron infusion (median, range)	7 days (1 to 85 days)	24 days (24 days)	6 days (1 to 85 days)	8 days (5 to 44 days)
Given iron infusion with ED OR HITH, n(%)	56 (71.8%)	29 (76.3%)	13 (68.4%)	14 (66.7%)
Discharged on oral iron, n(%)	22 (28.2%)	11 (28.9%)	3 (15.8%)	8 (38.1%)

HITH = Hospital in the Home, PRBC = packed red blood cells, LOS = length of stay, ED = emergency department

Drugs in blood: Is it safe to co-administer medications and red cell transfusions?

Charlewood R[1], Stone L[2]
[1]*New Zealand Blood Service, Auckland, New Zealand,* [2]*LabPlus, Auckland, New Zealand*

Aim: Current guidelines advise against co-administering intravenous medication with blood due to potential interactions of medications with blood, or additives in the unbuffered ex-vivo environment. Few drugs have been studied. This study aimed to evaluate haemolysis due to medications in a model mimicking co-administration through a 300ml/hour red cell transfusion line, using drugs commonly administered in anaesthetic or trauma settings.

Method: Samples from fresh whole blood and expired resuspended red cell units were mixed separately with twelve medications (rocuronium, suxamethonium, fentanyl, tranexamic acid, cefazolin, thiopentone, metaraminol, adrenaline, propofol, ketamine, midazolam, etomidate) at standard therapeutic concentrations. Samples were tested immediately, and following fifteen minutes' incubation at 37°C, along with calibrations and controls. Results were obtained for haemoglobin (Hb, g/L), LDH (U/L), free haemoglobin (mg/L) and agglutination and compared with the standard regulatory limit for haemolysis (0.8% by expiry).

Results: All drugs showed some haemolysis. Drugs showing <5% haemolysis (minimal) were fentanyl, ketamine, cefazolin, tranexamic acid. Drugs showing 5-15% (mild) were adrenaline, thiopentone, and midazolam but midazolam caused major haemolysis on delayed incubation. Drugs showing 15-25%(major) were metaraminol and suxamethonium. Only rocuronium caused complete haemolysis. Propofol and etomidate were unevaluable due to the opacity of their solutions.

Conclusion: This study presents a model for evaluating co-administration of drugs and blood via a flowing red cell transfusion line, facilitating study of other drugs. Some drugs clearly affect the red cells ex vivo, rocuronium in particular. Whilst the clinical significance of these findings is uncertain (given the small volume of blood involved) caution is advised. Suxamethonium may be safer to use than rocuronium when rapid sequence induction medications are co-administered with blood.

Beyond the surface: a tertiary centre investigation of transfusion reaction incidence and type

Wiltshire K, Garland R, Akers C, Pickles L, Davis A
[1]Alfred Health, Melbourne, Australia

Aim: To evaluate the safety of transfusion, impact of COVID-19 on transfusion adverse events, and identify contributors to adverse transfusion events with a goal to improve transfusion practice.

Method: A retrospective audit of reported transfusion reactions, and fresh blood component transfusions, between 1st January 2015 to 30th May 2023 was performed. Data was extracted from electronic laboratory systems, and Microsoft Excel spreadsheets originally maintained prospectively for auditing purposes. Types of transfusion reactions were compared with those reported by local serious incident reporting (STIR), national, and international haemovigilance programs.

Results: A total of 192,611 fresh blood components were transfused to 21,033 patients in the outpatient and inpatient setting. Out of the 732 reports of suspected transfusion reactions 82.79% were determined as incidences unrelated to transfusion. From the 124 confirmed transfusion reactions, 55 were reported to STIR. Rate of TACO was 0.11 per 1000 transfusions. Higher rate of transfusion reactions meeting STIR reporting requirements per 1,000 transfusions were observed in plasma transfusions during COVID (0.218 [2015-2019], 0.798 [2020-2021]). Acute haemolytic transfusion reactions made up 2% of transfusion reactions, one of which was an ABO incompatible transfusion. No transfusion related deaths were reported.

Conclusion: Total rate of transfusion reaction was 0.064%. However, incident rates of transfusion reaction were varied between the fresh blood components, with more serious transfusion reactions associated with platelets and plasma transfusions. While the overall rate of TACO was lower than expected compared to international reports, reactions reported to STIR were predominately allergic and TACO. The most serious preventable transfusion reaction reported was an ABO incompatible acute haemolytic transfusion reaction. Improvement should focus on addressing transfusion practices including appropriateness and preventive methods to improve patient safety.

Patient Blood Management Practices in paediatric patients undergoing craniosynostosis surgery.

Perchard M[1], Kinmonth A[1]
[1]*The Royal Children's Hospital, Flemington, Australia*

Aim: To review and understand Patient Blood Management (PBM) practices in patients undergoing craniosynostosis surgery and to determine the rate of alignment with the National Blood Authority (NBA) Patient Blood Management Guidelines: Module 2 Perioperative, and Module 6 Neonatal and Paediatrics.

Method: Retrospective electronic medical record review of 30 consecutive patients undergoing elective craniosynostosis surgery between October 2021 and June 2022 at the Royal Children's Hospital Melbourne. Review included pre and post-operative examination of ferritin and haemoglobin (Hb) to identify iron deficiency and anaemia and the use of iron replacement. Review also included the transfusion of blood products and intraoperative PBM practices.

Results: 93% (28/30) of patients had pre-operative blood samples collected that included a full blood count (FBC) and group and hold. Ferritin was not included for any patients. 6% (2/30) of patients were anaemic preoperatively with a Hb below reference range for age and gender. 93% (28/30) of patients were transfused with at least one blood product during surgery or in the post-operative period. 70% (21/30) of patients were anaemic on discharge with two patients discharged on oral iron therapy. Intraoperative practices included all patients received tranexamic acid and had their temperature recorded. When available, cell salvage was utilised.

Conclusion: PBM practices within craniosynostosis patients was varied. Pre-operative assessment of anaemia and iron deficiency could be improved by introducing routine ferritin testing.

Intra-operative practices generally reflected current PBM guidelines. Areas for improvement include use of cell salvage for all patients and ongoing education regarding restrictive transfusion practice. The rate of blood product transfusion in this cohort approaches 100% with majority of patients are anaemic at discharge. This identifies a potential group of patients that may benefit from a short course of oral iron therapy post operatively.

Development of a suite of state-wide tools to support safe transfusion practice with the introduction of an Electronic Medical Record

Catherwood A[1], Goodwin J[1], Grech J[1], Green A[1], Hunt R[1], O'Connor S[1], Bull K[1], Robinson K[1]
[1]BloodSafe, Adelaide, Australia

Aim: With progressive state-wide rollout of a single electronic medical record (EMR) across South Australian (SA) public hospitals, there was a need for specifically-tailored & consistent tools to educate staff & provide just in time reminders to ensure correct use of EMR functionality & safe workflows.

Method: SA BloodSafe Program worked with SA Pathology, Blood, Organ and Tissue Programs & state-wide EMR team to develop new tools & update existing resources.

Results: Transfusion related EMR educational resources developed & updated

Tool/Resource	Topic
Simulations/small group learning for medical students	• Creation of patients in EMR 'Play & Learn' sandbox • Safe transfusion sample ordering & collection - paper & EMR processes • Anaemia management & EMR prescribing of IV iron • Consent, prescribing & documentation of transfusion paper & EMR processes
eLearning	• Ordering & collection of blood group & antibody screen (G&S) - EMR functionality & workflows
Short videos & animation	• Videos & animation of ordering & collection of G&S - EMR functionality & workflows • Video of double independent checking of blood pack - EMR functionality & workflows
Lanyard Cards	• Collecting blood samples (with & without EMR) • Lanyard cards with QR codes to play short videos & animations (as above) of critical processes
Concise checklists	• Double independent checking of blood pack (including EMR specific functionality)
WBIT Reflection tool	• To help identify causative & contributing factors (including human factors) associated with WBIT incidents (with traditional & EMR processes)
Step by step illustrated guides to transfusion EMR functionality & workflows	• Prescribing & administering blood • Ordering & collecting transfusion samples

Conclusion: BloodSafe in collaboration with key partners have provided clinicians and students with concise, consistent & accessible tools to support safe transfusion practice during times of

changing workflows and cognitive overload associated with using EMR functionality, while reinforcing adherence to ANZSBT guidelines and critical patient ID processes.

O RhD negative red cell transfusions in small regional South Australian hospitals

Gore C[1], Fitzgerald L[2], Sinha R[1], Tocchetti R[1], Stathopoulos H[2], Ireland S[1]

[1]Blood, Organ & Tissue Programs, SA Health, Adelaide, Australia, [2]SA Pathology, Adelaide, Australia

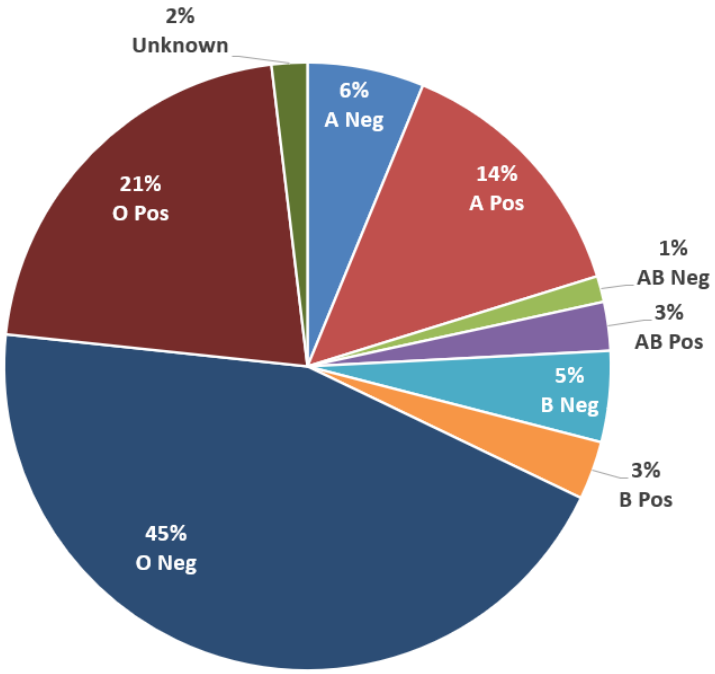
Aim: Regional South Australia (SA) has 62 hospitals. There are 26 hospitals with no on-site laboratory which hold 2 O RhD Negative (Neg) red cells (RC) for emergency use. These RCs are rotated to their partnering laboratories (regional and metropolitan). A retrospective audit was undertaken to examine emergency and elective transfusion of O Neg RCs in these regional hospitals, including the incidence of cross grouping.

Method: Using the Laboratory Information System, all consecutive RCs transfused to patients over a 2-year period (July 2020-June 2022) were included. Patient age, gender, and blood group together with product information (donor number, blood group, emergency, or crossmatch issue) were collected.

Results: During the 2-year period, a total of 3198 RCs were transfused. O RhD Positive (Pos) (1276 units, 39.9%), A Pos (930 units, 29.1%) and O Neg (579 units, 18.1%) constituted 87% of the total RCs transfused at these sites. Of the 579 O Neg RC, 457 (79%) were crossmatched and 121 (21%) were issued as emergency uncrossmatched RCs. Nearly 45% of the O Neg RCs were transfused to O Neg patients, the remaining were crossgrouped to patients with other blood groups (Figure 1). The emergency RCs were transfused to 73 patients, of which 40 (54.8%) were females. Of the 40 females transfused, 23 (31%) were less than 50 years of age.

Conclusion: Although emergency transfusion in regional South Australia was found to be uncommon, almost one-third were females less than 50 years. Low rates of emergency use of O Neg RCs led to a high incidence of cross grouping to O and A positive patients. Further analysis of red cell transfusion in the regional hospitals with onsite laboratories will help provide more insight around use of red cells and cross grouping in regional SA.

Figure 1. Patient Blood Group who received O Neg RC



GPIb-FilaminA interaction regulates megakaryocyte localization and budding during platelet biogenesis

Ellis M[1,2], Terreaux A[3], Alwis I[1,2], Smythe R[1,2], Perdomo J[4], Eckly A[5], Cranmer S[6], Passam F[1,2], Maclean J[1,2], Ruggeri Z[7], Lanza F[5], Taoudi S[3,8], Yuan Y[1,2], Jackson S[1,2,7]

[1]The Heart Research Institute, Newtown, Australia, [2]University of Sydney, Camperdown, Australia, [3]Walter and Eliza Hall Institute, Parkville, Australia, [4]Haematology Research Unit, St George and Sutherland Clinical School, Kogarah, Australia, [5]Université de Strasbourg, Strasbourg, France, [6]Eastern Health Clinical School, Monash University, Box Hill, Australia, [7]Department of Molecular Medicine, Scripps Research Institute, La Jolla, USA, [8]University of Melbourne, Parkville, Australia

Aim: Glycoprotein Iba is a critical receptor on the surface of platelets and megakaryocytes and is anchored to the membrane skeleton by Filamin A. While both GPIb and FlnA have well defined roles in platelet biogenesis, the critical nature of the interaction of these two proteins in megakaryocyte biology has not been evaluated previously *in vivo*. We therefore sought to determine the influence of disruption of the GPIba/FlnA interaction on *in vivo* platelet biogenesis, particularly focusing on the recently described phenomenon of megakaryocyte membrane budding.

Method: We generated a mouse model with either a wild-type (WT) or FlnA-binding mutant (FW) human GPIba transgene within a GPIba-null mouse. Platelet counts, platelet clearance, GPIba and GPIbb expression and proplatelet formation were assessed. We examined morphology of BM megakaryocytes using TEM. Megakaryocyte location, budding and cytoskeletal architecture were assessed using confocal and super-resolution microscopy from bone marrow cryosections.

Results: Mice expressing the mutant FW GPIba transgene exhibited a macrothrombocytopenia with platelet counts ranging from 150-200 x 10^6/mL with increased platelet volume. GPIb surface expression was preserved, albeit at lower levels than transgenic controls. Platelet clearance was normal and differentiation of FW megakaryocytes to proplatelets *in vitro* was preserved. Pulmonary proplatelets and bone marrow megakaryocytes numbers were normal in FW mice. The most striking defect in FW mice was the failure of normal DMS formation with dysregulated megakaryocyte budding, resulting in enlarged megakaryocyte buds that were ectopically released into the bone marrow interstitium. Expression of the cytoplasmic tail of GPIba in megakaryocytes corrected the macrothrombocytopenia by normalising bud size and megakaryocyte distribution.

Conclusion: These studies define a new mechanism of macrothrombocytopenia resulting from dysregulated megakaryocyte budding. The GPIba/FlnA interaction is dispensable for megakaryocyte budding, however it plays a major role in regulating megakaryocyte localization and budding morphogenesis.

Matrix Metalloproteinases as a Potential Therapeutic Target to Reduce Joint Damage in Haemophilic Arthropathy

Hauw W[1,2], Savvidou I[1], Vuong A[1], Cavallo I[1], Selan C[1], Nandurkar H[1,2], Sashindranath M[1,2]
[1]Australian Centre For Blood Diseases, Melbourne, Australia, [2]Alfred Health, Melbourne, Australia

Aim: Joint bleeding in people with Haemophilia leads to Haemophilic Arthropathy (HA) with devastating consequences. Matrix metalloproteinases (MMPs) are key players in inflammatory arthritis but their role in HA is still unclear. Critical for activation of MMPs are members of the fibrinolytic pathway uPA/tPA (urokinase and tissue plasminogen activator), and their target plasminogen. We aimed to characterise the role of MMPs and their associated pathways in HA development with a mouse model, to address the potential role of MMP inhibition as an adjuvant treatment for HA.

Methods: We simulated HA by causing 2 injuries in the knee joints of haemophilic mice over a 4-week period. Cartilage damage, iron deposition and synovial proliferation was studied using histology and immunofluorescence was used to characterise the inflammatory response. Gelatin zymography, western blotting and ELISA were used to assess MMP and plasminogen activation. The effect of MMP inhibition was studied by subcutaneous injection of Ilomastat (GM 6001; a pan MMP inhibitor;50mg/kg; 3 doses).

Results: Joint swelling was evident as early as 1 week, persisting to 4 weeks. We observed significant activation and upregulation of gelatinases MMP2and MMP-14 in injured knee joints compared to uninjured joints at 2- and 4-weeks post-injury. MMP14 activates MMP2, and MMP14 is induced by plasminogen activation. Accordingly, there was significant upregulation of uPA 2 weeks after knee injury. Administration of broad spectrum MMP inhibitor Ilomastat resulted in significant reduction of knee inflammation (knee diameter, IHC, IF) and cartilage damage (Fig 1).

Conclusion: The plasmin- MMP axis has a critical role in the development of HA. MMP blockade is a novel therapeutic approach that could complement current haemophilia management in mitigating joint damage.

Fig.1

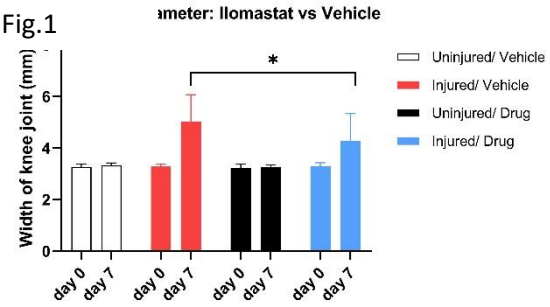


Fig 1: Knee joint diameters of mice receiving 2 weekly injuries monitored for 4 weeks were measured with electronic calipers on day of injury and one week post injury. Comparison is made between injured, uninjured joints and mice given 3 doses of pan-MMP inhibitor Ilomastat (Drug) post injury. Ilomastat significantly reduces joint swelling compared to mice given vehicle only (5% Dimethyl Sulphoxide/ 40% PEG300/ 5% Tween-80/ 50% saline). (n= 3 to 8 each group; *p<0.05)

Investigating the role of platelet chemokines in the development of acute lung injury

Fenwick J[1], Tedla N[2], Raftery M[2], Zhong L[2], Hiroshima Y[3], Geczy C[2], Passam F[1]
[1]University Of Sydney, Camperdown, Australia, [2]University of New South Wales , Kensington, Australia, [3]Tokushima University, Tokushima , Japan

Background: Platelets contain an array of chemokines in their alpha granules which are released upon activation. Two of these, S100A8 and platelet factor 4 (PF4), are chemoattractant, promoting neutrophil recruitment [1, 2]. However, under certain conditions, these chemokines exert anti-inflammatory properties; S100A8 administration to mice induced IL-10 production in the lungs protecting from acute lung injury (ALI) [3] whereas PF4 protects from acute liver failure [4]. The aim of this study was to investigate the role of S100A8 and PF4 in the development of ALI using a mouse model.

Methods: C57BL/6 mice were intranasally administered S100A8 prior to ALI induction by lipopolysaccharide (LPS). Mass spectrometry was performed on whole lung lysates. PF4 ELISA was performed on bronchoalveolar lavage fluid (BALF) to assess protein abundance. Lung tissues were collected and stained for platelet and PF4 spatial expression. Human platelets and umbilical vein endothelial cells were treated with LPS and S100A8 to determine platelet activation and adhesion respectively.

Results: Lung lysates showed a significant increase in PF4 content 6 hours after treatment with LPS and S100A8 versus LPS alone (3-fold $\log_2$ increase, $p<0.05$). This was accompanied by a decrease in the PF4 in the BALF 6 hours after treatment with LPS compared with LPS and S100A8 ($n=4$, $p<0.005$). LPS or S100A8 did not induce PF4 secretion or activation of human platelets. However, S100A8 increased the adhesion of platelets to endothelial cells ($n=3$, $p<0.05$).

Conclusion: We conclude that platelets are recruited within 6 hours after LPS stimulation to the lungs where they deposit PF4. S100A8 retains PF4 levels in the lung after LPS stimulation. This is likely due to the increased adhesion of platelets to lung endothelium. Further investigation is warranted to elucidate the underlying mechanisms and potential therapeutic implications of the PF4/s100A8 interaction.

1. Wong, S.W., et al., *Intranasal Delivery of Recombinant S100A8 Protein Delays Lung Cancer Growth by Remodeling the Lung Immune Microenvironment.* Front Immunol, 2022. **13**: p. 826391.
2. Chen, Y., et al., *Role of platelet biomarkers in inflammatory response.* Biomark Res, 2020. **8**: p. 28.
3. Hiroshima, Y., et al., *S100A8 induces IL-10 and protects against acute lung injury.* J Immunol, 2014. **192**(6): p. 2800-11.
4. Drescher, H.K., et al., *Platelet Factor 4 Attenuates Experimental Acute Liver Injury in Mice.* Front Physiol, 2019. **10**: p. 326.

Endoplasmic reticulum (ER) stress activation determines megakaryocyte and platelet ER chaperone distribution.

Kong Y[1,2,3], Ghani F[1], Fenwick J[1], Zhao H[1], Madsen S[4], Cooke K[4], Potter M[4], Stoeckli J[5], Morahan G[6], Cartland S[7], Kavurma M[7], James D[4], Passam F[1,2,3]

[1]Haematology Research Group, Charles Perkins Centre, University Of Sydney, Camperdown, Australia, [2]Central Clinical School, University of Sydney, Camperdown, Australia, [3]Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, Australia, [4]David James Lab, Charles Perkins Centre, University of Sydney, Camperdown, Australia, [5]Molecular Cell Biology, School of Life and Environmental Sciences, University of Sydney, Camperdown, Australia, [6]UWA Centre for Medical Research, University of Western Australia, Nedlands, Australia, [7]Vascular Complications Group, Heart Research Institute, Newtown, Australia

Aim: Platelet ER stress contributes to platelet activation and thrombosis and presents a potential target for antithrombotic therapy. Megakaryocyte endoplasmic reticulum (ER) stress has been shown to contribute to thrombopoiesis and thrombosis, but limited data exists on how disease states such as diabetes mellitus (DM) affects megakaryocyte ER stress. Additionally, whether ER stress leads to redistribution of prothrombotic ER-resident proteins, such as protein disulfide isomerase (PDI) and ERp5, to the platelet surface remains unknown.

Method: Femurs were isolated from murine models of type 1 DM with hyperlipidaemia (ApoE$^{-/-}$ ± streptozotocin) and type 2 DM (outbred mice on high fat diet). ER proteins were visualised by immunofluorescence microscopy in bone marrow cryosections. Healthy human donor platelets were treated with ER calcium mobilisers thapsigargin (TG) or 2,5-di-(tert-butyl)-1,4-benzohydroquinone (BHQ), and platelet surface CD62P, ERp5, PAC-1 and PDI were determined by flow cytometry. Platelet released proteins were analysed by Western blot.

Results: Activation of the inositol-requiring enzyme 1 (IRE1) pathway, an evolutionarily conserved ER stress pathway, was increased in diabetic megakaryocytes (Figure 1A and 1B). Increased PERK pathway activation, indicated by phospho-eIF2α, was only seen in megakaryocytes from the hyperglycaemic ApoE$^{-/-}$ mice (Figure 1A). Megakaryocyte ER stress was accompanied by upregulation of some ER chaperones, such as PDI, but not others, such as ERp5 (Figure 1A). Human platelets were activated after TG and tBHQ treatment. However, only TG treated human platelets demonstrated IRE1 pathway activation, and increased surface, but not released, PDI and ERp5.

Conclusion: We propose metabolic derangements such as hyperlipidaemia and hyperglycaemia can lead to megakaryocyte ER stress. This is accompanied by upregulation of some ER chaperones within the megakaryocytes. Importantly, ER stress appears to contribute to thrombosis by increasing platelet surface prothrombotic molecules, but not necessarily their secretion.

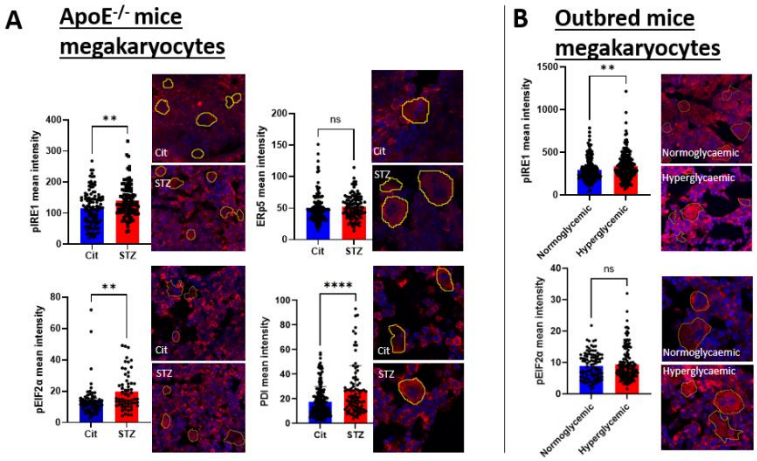


Figure 1 (left). ER stress pathways are differentially activated in murine models of A) hyperlipidaemic type 1 compared to B) type 2 DM. Megakaryocyte ER stress is associated with the induction of some but not all ER chaperones. ** indicates p of 0.001 to 0.01, **** indicates p<0.0001, Mann Whitney U test.

Endogenous FVIII activity and procedure-related FVIII use and bleeding: post hoc analysis of GENEr8-1

Pepperell D[1], Quon D[2], Wang J[3], Wang M[4], Park Y[5], Kenet G[6], Mahlangu J[7], Khoo T[8], Robinson T[9], Chavele K[9], Pipe S[10]

[1]Fiona Stanley Hospital, Murdoch, Australia, [2]Orthopaedic Hemophilia Treatment Center, Los Angeles, USA, [3]Center for Rare Disease and Hemophilia, , , [4]Hemophilia and Thrombosis Center, University of Colorado Anschutz Medical Campus, Aurora, USA, [5]Kyung Hee University Hospital at Gangdong, Seoul, South Korea, [6]The National Hemophilia Center, and Amalia Biron Research Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer, Tel Aviv University, Tel Aviv, Israel, [7]Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand and NHLS, Johannesburg, South Africa, [8]Haematology Department, Haemophilia Treatment Center, The Royal Prince Alfred Hospital, Sydney, Australia, [9]BioMarin Pharmaceutical Inc, Novato, USA, [10]Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, USA

Aim: GENEr8-1 (NCT03370913) is an ongoing phase 3 trial of valoctocogene roxaparvovec gene therapy

Method: In GENEr8-1, 134 male participants ≥18 years with FVIII ≤1 IU/dL on factor VIII (FVIII) prophylaxis (intention-to-treat [ITT] population) received an infusion of $6x10^{13}$ vg/kg valoctocogene roxaparvovec. The closest measurement to a procedure/bleed was identified. Procedures were identified as non-invasive or invasive; invasive were defined as major or minor based on common criteria.

Results: By the 2-year data cutoff, 260 total procedures were performed in 77 participants. Of 111 invasive procedures, 44 required FVIII treatment and 67 did not. Of 44 procedures performed with FVIII treatment, 11 were major (eg, joint debridement, arthrodesis) and 33 were minor (eg, dental extraction, biopsies). The 67 procedures performed without FVIII use were all minor invasive procedures. In the ITT population, mean (SD) FVIII activity at weeks 52 and 104 was 42.4 (45.3) IU/dL and 22.7 (32.8) IU/dL, respectively. At the closest measurement to minor procedures, mean (range) endogenous FVIII levels were 16.2 (<3–93.2) IU/dL for participants who required FVIII treatment and 50.5 (<3–255.7) IU/dL for those who did not. All 6 participants who underwent 11 major procedures received FVIII infusions regardless of endogenous FVIII activity and also required more mean (range) units of FVIII (255.4 [102.8–538.2] IU/kg) than those who underwent minor procedures (67.2 [13.7–324.3] IU/kg). There were 18 procedure-related bleeding episodes in 14 participants; 13 episodes required FVIII treatment and 5 did not. Mean (range) FVIII activity at the time was 60.4 (14.1–117.9) IU/dL for those not requiring treatment and 11.0 (<3–45.2) IU/dL for those requiring FVIII treatment.

Conclusion: In GENEr8-1, 67 of 111 invasive procedures in participants with severe Hemophilia A did not require FVIII treatment. FVIII use around minor procedures was associated with lower participant endogenous FVIII activity.

Fibrinolysis normalisation following cardiac bypass surgery: a prospective, observational study of recovery timeline and clinical associations.

Coupland L[1,2,3], Pai K[2], Pye S[2], Butorac M[2], Stewart A[2], Miller J[2], Aneman A[1,2,3]
[1]Ingham Institute Of Applied Medical Research, Liverpool, Australia, [2]Intensive Care Unit, Liverpool Hospital, Liverpool, Australia, [3]South Western Medical School, UNSW Medicine, Liverpool, Australia

Aim: Fibrinolysis, the natural process of blood clot breakdown, can be measured using rapid point-of-care viscoelastic testing with ClotPro® technology. Tranexamic acid is routinely given to mitigate hyperfibrinolysis during cardiac surgery using cardiopulmonary bypass and to minimise post-operative bleeding. The postoperative kinetics of fibrinolysis normalisation and its clinical associations are undetermined. The aim of this study, therefore, was to evaluate the kinetics of fibrinolysis following cardiac surgery with cardiopulmonary bypass, and the correlations to organ function, risk scores and length of stay in the Intensive Care Unit (ICU-LOS).

Method: A prospective, observational study was undertaken in 59 patients admitted to the ICU of Liverpool Hospital for postoperative care. Fibrinolysis was sequentially measured by the lysis time of the ClotPro TPA-test (TPA-LT). The regression analysis of TPA-LT over time split by short and extended ICU LOS was performed using LOESS smoothing with the standard deviation. Differences between groups on the first postoperative day were assessed by Mann-Whitney unpaired t-test. The associations between TPA-LT and biochemical markers of organ function, risk scores and ICU-LOS were determined using Spearman's correlation test, rho.

Results: In patients with a short ICU LOS (3 days, n=42), fibrinolysis normalised more rapidly than in patients requiring extended ICU aftercare (n=17), with a significant difference (p=0.008) observed from the first postoperative day. Impaired fibrinolysis correlated with creatinine (rho=0.67, p<0.001), lowest bicarbonate (rho=-0.42, p<0.01), urea (rho=0.47, p<0.001), Sequential Organ Failure Assessment score (rho=0.44, p<0.001), Acute Physiology and Chronic Health Evaluation III score (rho=0.47, p<0.001), Australia and New Zealand ICU Risk of Death score (rho=0.58, p<0.05), and ICU-LOS (rho=0.52, p<0.001).

Conclusion: Delayed normalisation of fibrinolysis in postoperative cardiac surgery patients is associated with organ dysfunction and extended need for intensive care support. Early identification of impaired fibrinolysis may be used to target interventions in patients at risk for postoperative complications.

Outcomes of FXa-inhibitor associated gastrointestinal bleeding: Do prohaemostatic agents change anything?

Ng S[1,2], Nguyen D[1], Hsu D[1,2]
[1]*NSW Health, Liverpool, Australia,* [2]*University of New South Wales, Sydney, Australia*

Aim: To determine whether the addition of prohaemostatic agents over anticoagulation cessation alone improves outcomes in patients with FXa-inhibitor associated gastrointestinal bleeding

Method: A retrospective analysis was performed between January 2015 to December 2021 of consecutive patients with FXa-inhibitor-related bleeding presenting within the South West Sydney Local Health District (SWSLHD). SWSLHD comprises of 6 acute public hospitals, servicing over 1 million residents (12% of NSW population).

Clinical and laboratory data points were retrospectively extracted through the SWSLHD electronic record system and blood bank database.

Results: 273 gastrointestinal bleeding events were identified, with an overall-in hospital bleeding-related mortality of 5.5%.

121 patients (44%) received prohaemostatic agents (plasma, Prothrombinex, FEIBA or a combination of these agents), with a bleeding-related mortality of 6.6%. Of these, 10/121 (8%) had anti-Xa levels <30ng/ml (whom received prohaemostatic agents prior to anti-Xa results). Of the 152 patients (56%) who did not receive prohaemostatic agents, bleeding-related mortality was 3.3%. There were a greater number of WHO grade 4 bleeds in the prohaemostatic group (22% vs 8%). There were no significant differences between the mean rivaroxaban and apixaban levels between the two groups.

128/273 patients (47%) were upper GI bleeds and 73% (94/128) of these underwent endoscopy within 24h – 83% in the prohaemostatic group and 64% in the non-prohaemostatic group. The median pre-endoscopy Rockall scores were 4 in both groups, with an endoscopy Rockall score of 6 in the prohaemostatic agent group versus 4 in the non-prohaemostatic group. The use of prohaemostatic agents did not appear to reduce the rate of recent haemorrhage.

Conclusion: The use of prohaemostatic agents in FXa-inhibitor associated bleeding does not appear to significantly influence the rate at which patients undergo endoscopy, nor significantly reduce the severity of gastrointestinal bleeding or bleeding-related mortality.

Spontaneous bleeding in chronic kidney disease patients - global coagulation assays may be predictive of bleeding risk.

Brook R[1,2], Ho P[1,2,3,4,5], Lim H[1,2,3,5]
[1]Department of Haematology, Northern Health, Epping, Australia, [2]Northern Pathology Victoria, Northern Health, Epping, Australia, [3]Australian Centre for Blood Diseases, Monash University, Melbourne, Australia, [4]Department of Medicine (Austin Health), University of Melbourne, Heidelberg, Australia, [5]Department of Medicine (Northern Health), Univeristy of Melbourne, Epping, 3076

Aim: The increased risk of bleeding seen in chronic kidney disease (CKD) has traditionally been attributed to platelet dysfunction but there is no simple blood test that can be used to predict this risk. We aim to explore if global coagulation assays (GCA), which provide a more wholesome assessment of coagulation, are predictive of risk of spontaneous major haemorrhage in CKD.

Method: Adult patients with CKD (defined by estimated glomerular filtration rate (eGFR) <30mL/min/1.73m^2) were recruited between March 2017 and July 2021 at Northern Health, Australia in this prospective observational study. Baseline clinical data and blood tests were collected at recruitment along with GCA including thromboelastography (TEG), overall haemostatic potential (OHP) and calibrated automated thrombogram (CAT). Patients on therapeutic anticoagulation or who did not have the complete suite of GCAs performed were excluded. The median follow-up was 4 years. Major bleeding is defined as per the guidelines of SSC-ISTH.

Results: A total of 87 patients were included in the study of which 65 (74.7%) patients were dialysis dependent. 67.8% were male (n=59), with median age 67 years (range 31-86). Ten spontaneous major bleeding events were captured (11.5%) with a rate of 3.00 per 100person-years. Spontaneous bleeding events were associated with a lower fibrinogen level (3.45 vs 4.85g/L, p=0.011), lower endogenous thrombin potential (ETP, 1099.6 vs 1340.0nM.min, p=0.013) and lower OHP (7.74 vs 17.0, p= 0.022). No significant association was demonstrated between bleeding and conventional coagulation testing (PT p= 0.922, APTT p=0.676), maximum amplitude on TEG (p=0.075), antiplatelet use (p=0.358), platelet count (p=0.124) or serum urea (0.289).

Conclusion: This study suggests that thrombin generation and OHP results may assist clinicians in prospectively identifying CKD patients at risk of developing spontaneous major haemorrhage. Furthermore, these results demonstrate that the increased bleeding risk associated with CKD may be more complex than previously assumed.

Endothelial-Targeted CD39 Ameliorates Acute Endothelial Toxin-Induced Pulmonary Hypertension in a Murine Model

Willcox A[1,2], Wang X[3], Selan C[1], Lee N[1], Calvello I[1], Bongcaron V[3], Walsh A[3], Song Y[3], Vuong A[1], Savvidou I[1], Peter K[2,3], Sashindranath M[1], Nandurkar H[1,2]
[1]ACBD, Monash University, Melbourne, Australia, [2]Alfred Hospital, Melbourne, Australia, [3]Baker Heart & Diabetes Institute, Melbourne, Australia

Aim: Pulmonary endothelial toxicity (by toxins, viruses (eg SARS-CoV-2) or bacterial sepsis) can lead to acute pulmonary vasculopathy leading to pulmonary arterial hypertension (PAH). In sepsis PAH is a predictor of mortality[1]. The actions of extracellular ATP and ADP released from damaged cells are mitigated by hydrolysis to adenosine monophosphate (AMP) by the ectonucleotidase CD39 (NTPDase1). AMP is further hydrolysed to adenosine by the ubiquitously expressed CD73. Pulmonary CD39 activity and adenosine signalling are disrupted in human PAH. We have developed a novel therapeutic '*anti-VCAM1-CD39*' localising the potent anti-inflammatory, vasodilatory and antithrombotic properties of CD39 to the inflamed microvasculature by binding to the receptor vascular cell adhesion molecule-1 (VCAM-1) expressed on activated endothelial cells. The aim of this work is to ascertain whether *anti-VCAM1-CD39* can prevent the development of acute toxin-induced PAH in a mouse model.

Method: PAH was induced in Balb/c mice with a single intravenous dose of Monocrotaline Pyrrole (MCTP, 8mg/kg), an endothelial toxin. Mice were then treated with *anti-VCAM1-CD39*, 0.4mg/kg 72 hours after MCTP injection or a control which included TBS (red), non-targeted CD39 (*iScr-VCAM1-CD39*, yellow) or VCAM-1 blockade alone (*anti-VCAM1*, purple). PAH was assessed using echocardiogram (pulmonary acceleration time vs pulmonary ejection time), right heart catheterisation (RVSP) and Fulton's index (Right Ventricle weight / Left Ventricle + septum weight) reflective of right ventricular hypertrophy.

Results: A single dose of *anti-VCAM1-CD39* (0.4mg/kg), at 72 hours when VCAM-1 is maximally upregulated, prevented the development of PAH (Figure 1). Furthermore, *anti-VCAM1-CD39* reduces small vessel remodelling and TNF-α, an inflammatory cytokine, and increases anti-inflammatory cytokine IL-10 at day 10. Preliminary data shows reduction in pulmonary CD39 in a murine model of SARS-CoV-2 infection.

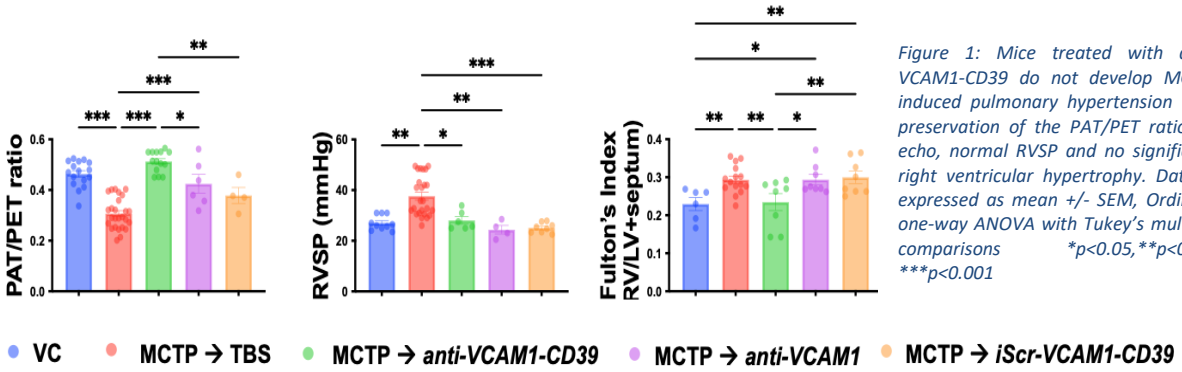


*Figure 1: Mice treated with anti-VCAM1-CD39 do not develop MCTP-induced pulmonary hypertension with preservation of the PAT/PET ratio on echo, normal RVSP and no significant right ventricular hypertrophy. Data is expressed as mean +/- SEM, Ordinary one-way ANOVA with Tukey's multiple comparisons *p<0.05,**p<0.01, ***p<0.001*

Conclusion: CD39 has emerged as an important molecule regulating inflammatory and coagulation homeostasis. Administration of endothelial targeted CD39 ameliorates the development of acute PAH providing potential as a novel therapeutic strategy.

1. Vallabhajosyula, S. *et al.* Doppler-defined pulmonary hypertension in sepsis and septic shock. *J Crit Care* 50, 201–206 (2019).

Patients with COVID-19 exhibit diminished thrombomodulin mediated inhibition of endogenous thrombin potential

Slocombe A[1,2], Baggio D[1], Zmijarevic M[1], Shugg C[1], Kelsey G[1], O'Mahoney M[1], Juneja S[1], Pasricha S[1,3,4]

[1]Diagnostic Haematology, The Royal Melbourne Hospital, Parkville, Australia, [2]Clinical Haematology, Eastern Health, Box Hill, Australia, [3]Clinical Haematology, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville , Australia, [4]Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

Aim: Endogenous thrombin potential (ETP) measured via thrombin generation assay (TGA) is a global coagulation assay sensitive to procoagulant and anticoagulant effects on thrombin generation, the latter via its interaction with thrombomodulin to activate protein C. We aimed to use TGA to demonstrate a hypercoagulable state in hospitalised COVID-19 patients compared to healthy controls.

Method: Fluorometric TGA (ThromboScreen, ST Genesia) was performed in 55 patients who presented with COVID-19 between July 2020 and February 2021, and 12 healthy controls using standard reagents (tissue factor/phospholipid, and tissue factor/phospholipid/thrombomodulin). Baseline demographics (age, sex) and coagulation profile (APTT, PT and fibrinogen) were collected for both cases and controls; additional disease characteristics (length of stay, intensive care admission, anticoagulant therapy, and d-dimer values) were recorded for COVID-19 patients. Between-group comparisons of ETP values were performed using *t*-tests.

Results: Among 55 COVID-19 patients, median age was 63 years, 29 (52.7%) were females and 49 (89.1%) received anticoagulation (44 prophylactic, 5 therapeutic). There was no significant difference in ETP between controls and COVID-19 patients (81.5% v 78.7% respectively, p=0.74), despite a high proportion of COVID-19 patients being on anticoagulation. There was a trend to higher ETP in the non-anticoagulated COVID-19 patients compared to controls (101.3% v 81.5% respectively, p=0.08). Baseline PT and APTT were not significantly different between controls and COVID-19 patients, although fibrinogen was elevated in COVID-19 patients (5.0 v 3.1, p=0.01). However, COVID-19 patients demonstrated significantly less thrombin inhibition by thrombomodulin than controls (561.4 v 320.8 nM/min respectively, p=0.03), including in the non-anticoagulated subgroup (633.7 v 320.8 nM/min, p=0.04).

Conclusion: Global coagulation assessment using fluorometric TGA demonstrates a hypercoagulable state in hospitalised COVID-19 patients characterized by impaired thrombin inhibition by thrombomodulin and normal ETP despite anticoagulation.

The efficacy of prohaemostatic agents in the management of FXa-inhibitor-related intracranial haemorrhages

Ng S[1,2], Nguyen D[1], Noori H[3], Hsu D[1,2]
[1]Haematology Department, Liverpool Hospital, Liverpool, Australia, [2]University of New South Wales, Sydney, Australia, [3]Radiology Department, Liverpool Hospital, Liverpool, Australia

Aim: To determine the efficacy of prohaemostatic agents in the management of FXa-inhibitor related intracranial haemorrhages.

Method: A retrospective analysis was performed between January 2015 to December 2021 of consecutive patients with FXa-inhibitor-related bleeding presenting within the South West Sydney Local Health District (SWSLHD). SWSLHD comprises of 6 acute public hospitals, servicing over 1 million residents (12% of NSW population).

Clinical and laboratory data points were retrospectively extracted through the SWSLHD electronic record system and blood bank database.

Results: 94 spontaneous intracranial bleeding events were identified, with an overall in-hospital bleeding-related mortality of 32%.

61 (65%) patients received prohaemostatic agents (plasma, Prothrombinex, FEIBA, or a combination of the three agents) with a bleeding-related mortality of 38%. Of these, 13/61 (21%) had anti-Xa levels <30ng/ml (whom received prohaemostatic agents prior to anti-Xa results). Of the 48/61 (79%) who received prohaemostatic agents appropriately (anti-Xa >30ng/ml), the bleeding-related mortality was 52%.

Of the 33 patients whom did not receive prohaemostatic agents, 10/33 had anti-Xa levels <30ng/ml. There were no bleeding-related deaths in this group. 23/33 had an anti-Xa level >30ng/ml with bleeding-related mortality of 39%.

36 patients had progress CT brain imaging within 24h of presentation.
Of these patients, despite 27/36 receiving prohaemostatic agents, 59% of patients had an increase in ICH volume, ranging from 0.7-109ml.
Of the 9 patients who did not receive prohaemostatic patients, 44% of patients had an increase in ICH volume, ranging from 3-39.5ml.

Conclusion: Patients who presented with clinically significant anti-Xa levels (>30ng/ml) had a higher risk of mortality that does not appear to change with the use of prohaemostatic agents. A delay in readily-available anti-Xa levels was associated with the inappropriate use of prohaemostatic agents in 21% of patients (total cost amounting to $48,858AUD).

Incidence of venous thromboembolism in patients with major burns receiving standard dose thromboprophylaxis: a retrospective study

Kay C[1], Bortz H[1], Poole S[1], Selvanayakam S[1], Tran H[1]
[1]*Alfred Health, Melbourne, Australia*

Aim: To evaluate thromboprophylaxis prescribing and incidence of venous thromboembolism (VTE) in patients with burns >20% total body surface area (TBSA).

Method: Retrospective cohort study conducted at a state-wide provider for adults with complex burns. Adult patients admitted between Jan-2019 and Oct-2021 with burns >20% TBSA were identified from the Victorian Adult Burns Service registry. Data retrieved from the registry and electronic medical records included demographic and clinical parameters (length of stay (LOS), %TBSA burns, thromboprophylaxis prescribed, dosing, time to administration, VTE). The primary outcome was the incidence of VTE. Data were analysed descriptively; chi-squared or Mann-Whitney U test determined statistical significance (p-value <0.05), comparing those with VTE to those without.

Results: Seventy-eight patients met inclusion criteria. Majority of patients were male (73.1%), median age was 42.5 years (IQR: 32.0-58.0) and LOS 24.4 days (IQR: 10.8-42.9); 3.8% of patients had history of thrombophilia and mortality rate was 24.4%. The median TBSA burns was 33.0% (IQR: 23.6-49.8), with a high proportion of patients (94.9%) experiencing a flame burn injury. All patients received pharmacological prophylaxis. Five patients (6.4%, 95% CI 2.1-14.3%) developed VTE whilst inpatients. Patients experiencing VTE were less likely to receive standard-dose VTE prophylaxis (40% vs 95%, P=0.0001), the time to first administration of VTE prophylaxis was longer (27.2 hours vs 23.7 hours, p=0.002) and they had a significantly increased LOS (90.9 vs 20.9 days, p=0.0002), compared to those who did not develop VTE. There were no differences in %TBSA.

Conclusion: A low incidence of VTE was identified in patients with major burns. There were statistically significant differences in time to first administration of thromboprophylaxis and optimal dosing of VTE prophylaxis, in patients with VTE compared to those without. Larger prospective studies will inform future guidelines on the use of increased dose thromboprophylaxis in patients with major burns.

Case-control study on dynamic upper limb ultrasound and biomarkers in idiopathic upper extremity deep vein thrombosis

Yuen H[1,2], Ali S[3], Tran H[1,2], Curry G[1], Vanhaltren K[1], Coombs P[1], Gardiner E[3], Chunilal S[1,2]
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia, [3]Australian National University, , Australia

Introduction: Idiopathic upper extremity deep vein thrombosis (IUEDVT) is postulated to arise from venous thoracic outlet syndrome (VTOS), where the subclavian vein is compressed intermittently upon arm abduction; resultant changes to vascular rheology may contribute to enhanced platelet activation. However, VTOS diagnostic criteria are lacking with no reference ranges for dynamic upper limb ultrasonography (DULUS) which assesses subclavian artery (SCA) compression with peak systolic velocity ratio (PSVR, abduction PSV expressed over neutral PSV) as a surrogate marker for venous compression.

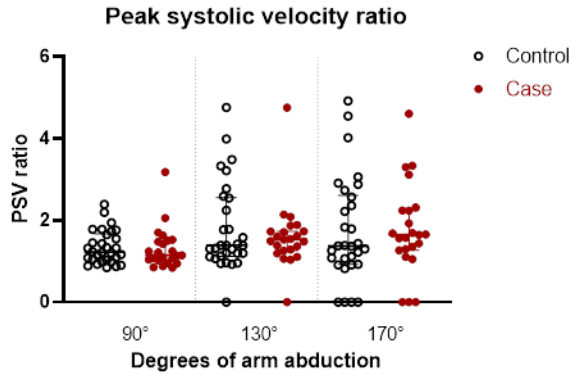
Aim: To establish a standardised protocol and reference intervals for DULUS and compare results in cases with controls, specifically SCA PSVR at 90° and 130° abduction. To compare D-dimer (fibrinolysis) and soluble glycoprotein VI (sGPVI; platelet activation) in cases and controls.

Method: Case-control study to establish DULUS reference ranges and compare results between 23 cases with antecedent IUEDVT treated with anticoagulation alone and 30 age- and sex-matched controls. Secondary outcomes included comparing D-dimer and sGPVI levels between groups. Non-gaussian distribution data are presented as median (interquartile range, IQR) and differences examined by Mann-Whitney test.

Results: Our 23 cases (median age 33.9 years, 39% male) were 26 months from IUEDVT diagnosis and received 7.1 months anticoagulation (range, 3 months-ongoing). Our controls were age- and sex-matched but lighter than cases. DULUS could not differentiate between cases and controls (Figure 1) and neither could D-dimer (0.08vs0.08mg/l, p=0.86) or sGPVI (8.2vs13.5ng/ml, p=0.1).

Conclusion: DULUS measurements including SCA PSVR at 90° and 130° abduction, D-dimer and sGPVI could not differentiate between IUEDVT and controls. Our findings challenge the utility of DULUS in VTOS diagnosis and the role of VTOS in the pathogenesis of IUEDVT.

Figure 1. Subclavian artery peak systolic velocity ratio at 90°, 130° and 170° abduction, erect, matched for arm dominance



Oral anticoagulant use in Australian atrial fibrillation patients with low risk of stroke: a national cohort studyt

Kefale A[1], Bezabhe W[1], Peterson G[1]
[1]***University of Tasmania, Sandy Bay, Australia***

Aim: The study aimed to describe the prescription pattern of oral anticoagulants (OACs) and its associated factors in atrial fibrillation (AF) patients with a low risk of stroke, using national data from Australian general practices.

Method: A retrospective cohort study was conducted using data collected from general practices enrolled in NPS MedicineWise, MedicineInsight program. We included patients with a recorded diagnosis of AF between 1 January 2011 and 31 December 2018 who had a CHA_2DS_2-VASc score of 0 for males or 1 for females. Patients were considered OAC users if there was a recorded OAC prescription within 60 days of their AF diagnosis. Predictors were assessed using logistic regression.

Results: The study included 2810 patients with low risk of stroke (62.3% males) with a mean age of 49.3 ± 10.8 years. Of the total cohort, 25.1% of patients were prescribed OACs within 60 days of AF diagnosis. Multivariable logistic regression found that female sex (adjusted odd ratio [AOR] 0.72; 95% confidence interval [CI] 0.60-0.86), higher socioeconomic status (AOR 0.66; 95% CI 0.48-0.89 for SEIFA quintile 2, AOR 0.76 ; 95% CI 0.57-1.00 for quintile 3, AOR 0.71; 95% CI 0.53-0.95 for quintile 4 and AOR 0.72, 95% CI 0.54-0.96 for quintile 5 compared to SEIFA quintile 1), presence of chronic liver disease (AOR 0.27; 95% CI 0.08-0.91) and depression (AOR 0.80; 95% CI 0.65-0.99) were negatively associated with OAC prescription. Increasing patients' age (AOR 1.03; 95% CI 1.02-1.04) and the year of diagnosis (2015-18 compared to 2011-12, AOR 1.79; 95% 1.30-2.46) were positively associated with OAC prescription.

Conclusion:
One in four patients with AF received OAC therapy despite a low risk of stroke. Sex, age, socioeconomic status, depression, chronic liver disease, and diagnosis period were the key determbolesinants of OAC prescription in the low-risk patients.

Perioperative management with efanesoctocog alfa in patients with hemophilia A in the phase 3 XTEND-1 study

Klamroth R[1], von Drygalski A[2], Hermans C[3], Santagostino E[4], Dumont J[5], Abad-Franch L[4], Willemze A[6], **Khoo L[7]**

[1]Vivantes Klinikum, Friedrichshain, Berlin, Germany, [2]Division of Hematology/Oncology, Department of Medicine, University of California, San Diego, San Diego, USA, [3]Haemostasis and Thrombosis Unit, Division of Haematology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium, [4]Sobi, Basel, Switzerland, [5]Sanofi, Cambridge, USA, [6]Sanofi, Amsterdam, Netherlands, [7]Institute of Haematology, Royal Prince Alfred Hospital, Sydney, Australia

Aim: The Phase 3 study, XTEND-1 (NCT04161495), showed once-weekly efanesoctocog alfa provided superior bleed prevention to prior FVIII prophylaxis and was well tolerated in adults/adolescents with severe haemophilia A. Here, we report the efficacy and safety of efanesoctocog alfa for perioperative management in patients who underwent major surgery in XTEND-1.

Methods: Patients on prior FVIII prophylaxis entered Arm A (52 weeks once-weekly efanesoctocog alfa [50 IU/kg]). Patients receiving prior on-demand therapy entered Arm B (26 weeks on-demand efanesoctocog alfa [50 IU/kg], then 26 weeks once-weekly prophylaxis). Patients in the surgery subgroup were to receive a preoperative 50 IU/kg dose, followed by 30 or 50 IU/kg every 2–3 days, as needed. Secondary endpoints included number/dose of efanesoctocog alfa injections, hemostatic response assessment, factor consumption, estimated blood loss, and number/type of blood transfusions during the perioperative period.

Results: Eleven patients (10 Arm A; 1 Arm B) underwent 12 major surgeries while receiving efanesoctocog alfa; 6 were orthopedic. Hemostatic response was rated excellent for all surgeries. Eleven surgeries required 1 preoperative efanesoctocog alfa injection; median (range) dose was 49.9 (12.7–51.7) IU/kg. For 1 surgery during prophylaxis, no preoperative dose was reported. Median (range) consumption was 31.8 (24.3–103.0) IU/kg for Day 1–3 and 103.20 (95.5–206.1) IU/kg for Day 4–14. Median (range) number of injections was 1.0 (1–2) for Day 1–3 and 2.0 (2–4) for Day 4–14 (n=11). Median (range) estimated blood loss was 75 (0–500) mL during surgery (n=6). During the surgical period, no blood transfusions were required and no treatment-emergent serious adverse events reported.

Conclusion: Efanesoctocog alfa was effective and well tolerated for the perioperative management of adults/adolescents with severe hemophilia A who underwent major surgery. One preoperative injection was sufficient for the surgery and all hemostatic responses were rated excellent.

Investigating the bidirectional interactions between platelets and colorectal cancer and the effects of aspirin

Capraro D[1,2], Connor D[1,2], Joseph J[1,2,3]
[1]Blood, Stem Cell and Cancer Research Program, St Vincent's Centre For Applied Medical Research, Darlinghurst, Australia, [2]St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, Australia, [3]Department of Haematology, St Vincent's Hospital Sydney, Darlinghurst, Australia

Aim: It is well established that platelets and cancer have a complex and interconnected relationship. There is also evidence that aspirin can reduce colorectal cancer (CRC) incidence, metastasis, and mortality. We hypothesise that the anti-cancer effect of aspirin may derive from its anti-platelet effect.

Method: Whole blood and platelets from healthy controls (n=5) and patients with metastatic CRC (n=3) were incubated with or without aspirin before co-incubation with two CRC cell lines (HCT15 and HCT116). Platelet activation was assessed using flow cytometry by measuring CD62P and CD63 expression and PAC-1 binding. Platelet binding to CRC cell lines was examined with immunofluorescence microscopy. The effect of platelets and platelet releasate on the proliferation of CRC cell lines was investigated using CFSE and Transwell assays assessed migration and invasion. Paired data was analysed using a Wilcoxon matched-pairs signed rank test and unpaired data was analysed with a Mann-Whitney test.

Results: CRC cell lines activated platelets from healthy controls and patients in a dose-dependent manner. There was no difference in the levels of platelet activation between controls and patients and aspirin had no effect on platelet activation. Platelets from controls and patients bound to CRC cell lines and aspirin had no effect on platelet binding. Platelets and platelet releasate from both controls and patients had no effect on the proliferation of CRC cell lines. Platelets and platelet releasate, particularly from patients, increased the migration and invasion of CRC cell lines but this effect along with any inhibition by aspirin was not statistically significant.

Conclusion: This study demonstrated that CRC cell lines activate platelets, confirming there are bidirectional interactions between platelets and CRC cells however aspirin appears to have no effect in this *in vitro* setting. This study provides a foundation for further mechanistic investigations into the role of platelets in cancer.

Activated Coagulation Factor XII (Factor XIIa): A unique target for in vivo molecular imaging

Walsh A[1,2,3], Yu E[1,2,3], McFadyen J[2,3,4,5], Bongcaron V[1,2], Moon M[2,5], Huang A[2], Arthur J[6], Muir I[6], Rayzman V[6], Panousis C[6], Wang X[1,3,5,7], Peter K[2,3,5,7]
[1]Molecular Imaging and Theranostics Laboratory, Baker Heart And Diabetes Institute, Melbourne, Australia, [2]Atherothrombosis and Vascular Biology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia, [3]Department of Medicine, Monash University, Melbourne , Australia, [4]Clinical Haematology Department, Alfred Hospital, Melbourne , Australia, [5]Department of Cardiometabolic Health, University of Melbourne, Melbourne , Australia, [6]CSL Limited, Bio21 Institute, Parkville, Melbourne , Australia, [7]Department of Cardiovascular Research, Translation and Implementation, La Trobe University, Melbourne , Australia

Aim: Current clinical imaging of thromboembolic diseases often relies on indirect detection of thrombi, which may delay diagnosis and treatment. Therefore, the development rapid, specific, and direct imaging of thrombi using molecular imaging is highly sought-after. Coagulation factor (F)XIIa, which initiates the intrinsic coagulation pathway but is dispensable for normal hemostasis, is therefore an ideal target for diagnostic and therapeutic approaches.

Method: An FXIIa-specific antibody, 3F7, was conjugated to a near-infrared (NIR) fluorophore. Acute thrombosis was induced in the right carotid artery of mice by $FeCl_3$ injury, while the left vessel served as a healthy control (n=6). Targeted or non-targeted probe was administered intravenously and circulated for 1 hour before *in vivo* imaging by 3D fluorescence emission computed tomography (FLECT)/CT. The vessels were then excised for *ex vivo* IVIS imaging. Pulmonary embolism was induced by intravenous injection of thromboplastin and fluorescent probe before lungs were excised for *ex vivo* IVIS imaging (n=4-5).

The Mann–Whitney test for two non-parametric groups, and one-way ANOVA with Tukey's test for more than two parametric groups with one independent variable were used for statistical analysis.

Results: We detected probe binding to carotid thrombosis with 3D FLECT/CT *in vivo*, and measured a significant fold increase in signal between healthy and control vessels from mice injected with 3F7-NIR compared to non-targeted probe (p=0.002) *ex vivo*.

We demonstrated *ex vivo* imaging of thrombi pulmonary embolism, measuring increased NIR signal in lungs from mice injected with 3F7-NIR compared to mice injected with non-targeted probe (p=0.0008) and healthy lungs from mice injected with 3F7-NIR (p=0.021).

Conclusion: We demonstrate that FXIIa targeting is highly suitable for the specific detection of venous and arterial thrombi. This approach will allow direct, specific, and early imaging of thrombosis in preclinical imaging modalities, and may facilitate monitoring of anti-thrombotic treatment *in vivo*.

Cancer-related cognitive impairment in patients with newly diagnosed aggressive lymphoma compared to healthy controls: An exploratory study

Gates P[1,2,3], Dhillon H[4], Wilson C[2,3,5], Krishnasamy M[1,3,6], Gough K[1,3]
[1]Peter MacCallum Cancer Centre, Parkville, Australia, [2]Austin Health, Heidelberg, Australia, [3]University of Melbourne, Parkville, Australia, [4]University of Sydney, Camperdown, Australia, [5]La Trobe University, Heidelberg, Australia, [6]Victorian Comprehensive Cancer Centre Alliance, Melbourne, Australia

Aim: Cancer-related cognitive impairment is a recognised adverse consequence of cancer and its treatment but there is little research including patients with aggressive lymphoma. The aim of this study is to describe self-reported cognitive function and neuropsychological performance in a lymphoma population and compare their function and performance with healthy controls. We also examine the associations between patients' neuropsychological performance, cognitive function and distress.

Method: Secondary analysis of data from a longitudinal feasibility study of 30 patients with newly diagnosed aggressive lymphoma, and a cohort study that included 72 healthy controls was undertaken. Patients completed self-report measures and neuropsychological tests before and 6-8 weeks after chemotherapy, including the PROMIS Anxiety 7a/Depression 8b and FACT-Cog; and the Trail Making Test, Hopkins Verbal Learning Test, and WAIS-R Digit Span. Healthy controls completed the FACT-Cog and neuropsychological tests at study enrolment and six months later. Mixed models were used to analyse FACT-Cog and neuropsychological test scores. Kendall's Tau provided a measure of association between global deficit scores and scores from other measures.

Results: Patients and healthy controls were well matched on key demographic variables. Most differences between patients' and healthy controls' neuropsychological test scores were large-sized; the performance of patients was worse both before and after chemotherapy (most $p<0.001$). The same pattern of results was observed for the impact of perceived cognitive impairment on quality-of-life (both $p<0.001$), but not perceived cognitive impairment or abilities (all $p>0.10$). Associations between neuropsychological performance, self-reported cognitive function and distress were trivial to small-sized (all $p>0.10$).

Conclusion: For many patients with aggressive lymphoma, impaired neuropsychological test performance and the impact of perceived impairments on quality-of-life precede chemotherapy and are sustained 6-8 weeks after chemotherapy. Our data support the need for further longitudinal studies in this population to inform development of targeted interventions to address cognitive impairment.

"No conflict of interest to disclose".

The Steroid Symptom Questionnaire for Multiple Myeloma (SSQ-MM) validated for clinical use.

King T[1,2], Jagger J[5], Rutherford C[2,3], Acret L[2,3], Tait M[2,3,4], Sipavicius J[7], McCaughan G[6], Stapleton S[6], White K[2,3,4]

[1]Institute of Haematology, Royal Prince Alfred Hospital, , Australia, [2]Cancer Care Research Unit, Faculty of Medicine and Health, The University of Sydney, , Australia, [3]The Daffodil Centre, The University of Sydney, a joint venture with Cancer Council NSW, , Australia, [4]Sydney Local Health District, , Australia, [5]Cancer Services, Central Coast Local Health District, , Australia, [6]Department of Haematology, St Vincent's Hospital, Sydney, , Australia, [7]Department of Haematology, Royal North Shore Hospital, , Australia

Aim: Steroids used to treat multiple myeloma (MM) can cause significant side effects. We developed the first Patient Reported Outcome Measure (PROM)to monitor steroid side effects in this population: the SSQ-MM. Pilot testing demonstrated high feasibility, acceptability, and internal consistency. We aimed to further test clinical validity and reliability.

Method: This multi-centre, cross-sectional study administered the SSQ-MM and two EORTC PROMs (QLQ-C30 & QLQ-MY20) to participants recruited across four Sydney hospitals, with the SSQ-MM repeated 1-week later. An estimated 200 patients are required for all planned analyses. Internal consistency reliability was tested with Cronbach's alpha >0.8 for individual patient decision-making. Test-retest using intraclass correlation coefficient >0.7 tested scale stability reliability.

Results: To date, 109/200 patients aged 45-86 yrs, 57% male, were recruited with average 4 yrs (range, 1mth to 22yrs) since diagnosis. Average dexamethasone dose was 28mg weekly (4 to 80mg), or 124mg per cycle (12 to 180mg). Common regimens were Lenalidomide & Bortezomib (26.6%), Lenalidomide (22%) and Daratumumab & Bortezomib (13.8%). Cronbach's alpha at time points 1 (0.83) and 2 (0.84) showed internal consistency, and test-retest indicated the scale was stable (ICC: 0.844, 95%CI: 0.779 to 0.892; p<0.001). Frequently reported symptoms were disturbed sleep (91.7%), fatigue (80.7%), and trouble concentrating (79.8%). Patients typically reported 10 symptoms concurrently (1 to 19), with 4 symptoms rated as severe (0 to 12). Frequently reported severe symptoms included disturbed sleep (67%), fatigue (50.5%), and fragile skin (45.9%), with disturbed sleep rated as most bothersome (48.6%).

Conclusion: The SSQ-MM currently demonstrates high levels of feasibility, acceptability, and reliability and is suitable for clinical use. The study continues to evaluate convergent and discriminant validity by exploring relationships between similar and dissimilar constructs within EORTC QLQ-C30 and QLQ-MY20, enabling the SSQ-MM to be used in future clinical trials of steroid treatments for MM.

Objective measures of sleep disturbances in patients receiving steroids for the treatment of multiple myeloma: Findings from a pilot study.

King T[1,2], Melehan K[3,4], Gauthier G[3], Boustany C[2,5,6], Acret L[2,5], White K[2,5,6]
[1]Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, Australia, [2]Cancer Care Research Unit, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia, [3]Department of Respiratory and Sleep Medicine, Royal Prince Alfred Hospital, Camperdown, Australia, [4]Sleep Medicine, The University of Sydney , Camperdown, Australia, [5]The Daffodil Centre, The University of Sydney, a joint venture with Cancer Council NSW, Sydney, Australia, [6]Sydney Local Health District, Sydney, Australia

Aim: Glucocorticosteroids (steroids) have multiple deleterious side effects (SE). Disturbed sleep is the most frequently reported SE, identified in up to 95% of patients (King 2019). This pilot study, aimed to characterise the sleep disturbances associated with steroids taken as treatment for MM using actigraphy.

Method: 10 MM participants currently taking steroids for MM, with associated disturbed sleep were recruited. Sleep duration, sleep efficiency and wake bouts were measured over three weeks of monitoring using actigraphy, a validated method of objective sleep monitoring using a watch-like device, and sleep diaries to document subjective sleep experience. Actigraphy data was analysed in a blinded fashion, utilising light, and activity levels to determine attempted sleep periods, with sleep diaries to confirm these. Semi-structured interviews were also undertaken.

Results: To date 8 participants have been recruited, 4 male and 4 female. Participants were median of 60.5 (53-71) years of age, receiving median of 100 mg (24-160) of dexamethasone per cycle of treatment and had received up to 4 lines of previous treatment. We report actigraphy data on 3 participants with completed data. Sleep duration was reduced in 2/3 of participants by 33%, and 43% on dexamethasone nights. There was a reduction in average sleep efficiency (% time asleep) and a lowering of wake bouts with dexamethasone, 83.0% vs 75.3%, and 29.5 vs 21.0, respectively. The average fragmentation index (sleep disturbance) slightly increased from 17.2 to 19.8 on dexamethasone nights.

Conclusion: This is the first study to objectively characterise sleep disturbances associated with dexamethasone treatment in MM. Our preliminary results indicate a reduction in sleep duration and sleep efficiency on nights participants took dexamethasone. Sleep was more disturbed on these nights, with less, though longer, periods of wakefulness. Data on the full cohort is pending and will be further informed by the qualitative interviews.

Reference: King T, Jagger J, Fethney J, Boustany C, Joshua D, White K (2019). The Steroid Symptom Questionnaire Multiple Myeloma (SSQ-MM): Feasibility, acceptability, reliability and internal consistency. Clinical Lymphoma, Myeloma and Leukemia, 19(10): e341-e341

Nurse Practitioner blood product prescribing in malignant haematology and bone marrow transplantation, a single centre experience

Vassili C[1], Cirone B[1], Lincoln M[1], Kliman D[2], Tiong I[1], Wong S[1], Tam C[1], Kenneally A[1]
[1]Alfred Health, Melbourne , Australia, [2]Royal North Shore Hospital , St Leonards , Australia

Background: Nurse practitioner (NP) blood prescribing is a niche concept in Australia. There is no nationally recognised credentialing program, and there is limited data about NP blood prescribing practices. An opportunity to enhance the care of patients with haematological malignancy requiring transfusion support within the Cancer outpatient setting at Alfred Health was recognised by enabling independent red blood cell (RBC) and platelet prescribing by NPs. There are four endorsed NPs in the Malignant haematology and bone marrow transplantation service.

Aim: To describe the NP blood prescribing credentialing program and present the audit activity data.

Method: A working group was formed in May 2020 to develop a framework for credentialing of NP blood prescribing. This was based on the *Nurse Practitioners prescribing and blood products* guidance document by Blood Matters[1]. Assessment for competence requires the completion of self-directed BloodSafe[2] and Lifeblood Transfusion[3] online modules, and supervised learning with a medical mentor utilising the portfolio of evidence in the guidance document[1]. The credentialing program was endorsed by the Nursing Scope of Practice and Transfusion Committees in October 2020.

Results: Three NPs have completed the credentialing program since January 2021. Six months of prospective prescribing data using a Department of Health audit tool[4] was recorded and independently assessed by each medical mentor. Collectively there were 139 episodes of blood prescribing; 141 units of RBC and 56 units of platelets. One adverse platelet transfusion reaction was documented. All prescribing episodes were deemed clinically appropriate by the medical mentors. The audit was presented to the Transfusion Committee.

Conclusion: Alfred Health is committed to ensuring Patient Blood Management is an integral part of patient care. Organisational governance and medical mentor support was essential in the NP credentialing process. The NPs have regularly and appropriately utilised this extension of scope of practice, benefiting patients requiring transfusion support in the outpatient setting.

References:

1 *Nurse practitioners prescribing blood and blood products, guidance document.* Available at https://www.health.vic.gov.au/sites/default/files/2021-11/nurse-practitioners-prescribing-blood-and-blood-products.pdf
2 *BloodSafe Elearning* Australia. Available at: https://bloodsafelearning.org.au/
3 *Learn Lifeblood.* Available at: https://www.lifeblood.com.au/health-professionals/learn
4 Department of Health Victoria. (2021). *Data Collection Tools, Department of Health. Victoria, Australia.* Available at: https://www.health.vic.gov.au/patient-care/data-collection-tools

Strengthening communication, education and support for consumers of allogeneic stem cell transplant by experiences of care surveys

Cibich A[1], Stokes C[1], Ward M[1], Bubner K[1], Munt R[1,2], Cameron K[1,2], Bardy P[1], **Loft N[1]**
[1]Royal Adelaide Hospital, Adelaide, Australia, [2]University of Adelaide, Adelaide, Australia

Introduction: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is a highly specialised and costly procedure. Complications are common and serious, resulting in ongoing interaction with healthcare systems. Current evidence reports significant decline in patient quality of life and distress in carers[1,2]. Experiences of care surveys (ECS) are commonly implemented in cancer care, however, omit important facets unique to alloHSCT. Additionally, these surveys do not engage carers. Utilising an *alloHSCT-centric* ECS to engage patients *and* carers, we aimed to identify gaps in service delivery and embed consumer feedback into standard of care.

Methodology: Purposive sampling was used to recruit previous alloHSCT patients and their carers. Data were collected using an adapted *alloHSCT-centric* ECS[3] including questions on before, during and after transplant. Quantitative data were analysed using descriptive statistics and qualitative data via Braun and Clark's thematic analysis framework[4].

Findings: 42 patients and 29 carers completed the survey. Core themes identified across all phases of transplant were ***communication, education,*** *and* ***support***. Before transplant, 63% of patients and 69% of carers reported the explanation of side-effects was understandable, however only 21% and 29% of patients and carers reported so after transplant. 95% and 79% of patients and carers reported having a main contact before transplant in the healthcare team, but only 41% and 39%, respectively post-transplant. Most patients and carers reported that they felt supported during and after transplant, however expressed the need for peer support.

Key recommendations include developing standardised, multi-media educational resources to integrate with newly implemented electronic communication systems, accessible across sites and digitally on mobile devices. We will continue to engage consumers and collaborate with allied health services to bridge knowledge gaps, in particular late side-effects. It is intended in future to seek feedback during the 3 phases of transplant so issues can be identified and addressed at the time.

Conclusion: These survey findings help consumer experiences through identifying opportunities for continued, targeted education, communication, and support to enable early intervention and continuity of care across all phases of allogeneic transplant.

References

- El-Jawahri, A.R., Vandusen, H.B., Traeger, L.N. et al, (2016), *Quality of life and mood predict posttraumatic stress disorder after hematopoietic stem cell transplantation*. Cancer, 122: 806-812. https://doi.org/10.1002/cncr.29818

1. Beattie, S. and Lebel, S. (2011), *The experience of caregivers of hematological cancer patients undergoing a hematopoietic stem cell transplant: a comprehensive literature review*. Psycho-Oncology, 20: 1137-1150. https://doi.org/10.1002/pon.1962
2. *National Cancer Patient Experience Survey* (2015), CROS - Collaboration in Research and Methodology for Official Statistics, viewed 24th May 2022, https://ec.europa.eu/eurostat/cros/content/national-cancer-patient-experience-survey_en
3. Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. Qualitative Research in Psychology, 3(2), 77–101. https://doi.org/10.1191/1478088706qp063oa

Developing a national support and information program for younger people living with myeloma in Australianur

Smith N[1,2], Keogh J[1], Bowley C[1,4], Jones L[1,5], Beer H[1,3], Combe N[1,3]
[1]Myeloma Australia, Cottesloe, Australia, [2]Cancer Network WA, Nedlands, Australia, [3]Peter MacCallum Cancer Centre, Melbourne, Australia, [4]Central Coast Local Health District, NSW Health, Central Coast, Australia, [5]St Vincent's Public Hospital, Melbourne, Australia

Introduction / Aim: Myeloma is an incurable cancer of the plasma cells that most commonly presents in older people, however, there is a small number of people diagnosed between the ages of 25 and 45, often with young families and work commitments, who need to live through the multiple relapses and remissions often experienced in myeloma. The aim of this innovation was to better understand the specific needs of younger people with myeloma in Australia and to develop and refine a suite of resources aimed at support, education, and connection for this group.

Method: We invited people identifying as a younger person or their support person to complete an online survey about being a younger person living with myeloma. The survey consisted of 21 questions focused on current supports available and their suitability, the experience of being a younger person with myeloma, and unmet support and information needs.

Results: 105 surveys were completed with regional and metropolitan representation. Perspectives shared in the survey identified issues and needs, including parenting and relationship challenges, communication with children, navigating work, future planning, treatment late effects, employment, and accessing practical assistance. Current support programs were evaluated by participants. Results informed the existing national younger person's support group and the recording of the two podcasts involving a young person with myeloma. A comprehensive, myeloma-specific younger person's written resource is being developed and a national webinar is planned to address some of the identified needs.

Conclusion: This project has allowed younger people with myeloma to share their insights and experiences and guided the development of resources. Future plans include an evaluation survey, and focus groups aimed at gaining a deeper understanding of the younger persons experience, to inform the planning of future events, resources and supports for younger people with myeloma in Australia.

A scoping review of CD20-CD3 bispecific antibody adverse events to inform nursing practice in B-cell non-Hodgkin Lymphoma.

van der Linde S[1], Knights E[2], Robertson M[1]
[1]*Peter Maccallum Cancer Centre, Parkville, Australia,* [2]*Barwon Health, Geelong, Australia*

Aim: To provide evidence-based guidance to cancer nurses by reporting the common adverse events (AEs) associated with T-cell redirecting bispecific-antibody (BsAb) treatment in patients with B-cell non-Hodgkin Lymphoma (B-NHL). Secondary objectives were to describe the symptoms, incidence, severity, timing and onset of cytokine release syndrome (CRS), highlighting differences across agents.

Method: Databases were systemically searched for prospective interventional clinical trials of CD20-CD3 BsAbs reporting safety data for B-NHL patients published between January 2013 to March 2023. Multisite collaboration of specialist lymphoma nurses with practice experience in BsAb delivery and evidence from the literature was used to generate guidance for safe patient management (Figure 1).

Results: 1073 publications were identified. Safety data was extracted from 84 records to report the most common AEs and management (Table 1). Six BsAbs were identified: epcoritamab, glofitamab, imvotamab, mosunetuzumab, odronextamab and plamotamab. CRS was the most common AE reported, followed by neutropenia. No studies reported nurse led interventions, demonstrating a lack of standardised guidelines for nurses administering these BsAbs. CRS primarily occurred during the first cycle and was mostly low-grade, however onset time and duration differed across agents. Mitigation strategies for CRS included premedication with corticosteroids, antipyretics and antihistamine, step-up dosing, and planned hospitalisations. Common signs and symptoms of CRS were pyrexia, chills, tachycardia and hypotension. Supportive management, tocilizumab and corticosteroids were used for the treatment of CRS.

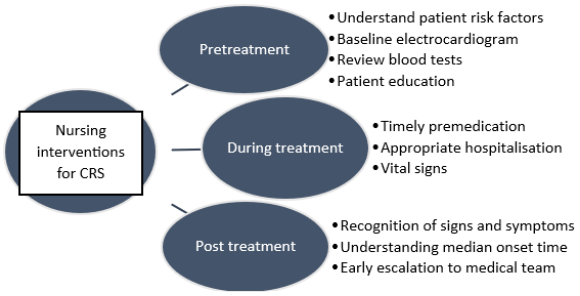
Conclusion: BsAbs represent an effective treatment option in B-NHL with a unique side-effect profile. Some agents are now available through compassionate access programs and will be increasingly encountered in routine practice. This is the first review to synthesize evidence from clinical trials of CD20-CD3 BsAb for nursing practice, informing nurses of the most common AEs and nursing led interventions for safe delivery.

Table 1. Common AEs reported in single agent bispecific antibody treatment in R/R B-NHL

	Epcoritamab	*Glofitamab*	*Imvotamab*	*Mosunetuzumab*	*Odronextamab*	*Plamotamab*
Clinical Trial ID	NCT03625037	NCT03075696	NCT04082936	NCT02500407	NCT02290951	NCT02924402
Author	Thieblemont et al., 2023	Hutchings et al., 2021	Budde et al., 2021	Budde et al., 2022	Bannerji et al., 2022	Patel et al., 2022
Frequency of AEs						
Pyrexia	23.6%	12.9%	25%	NR	73%	38.9%
Neutropenia	21.7%	25.1%	25%	28.4%	NR	NR
CRS	49.7%	50.3%	NR	27.4%	61%	25%
Characteristics of CRS						
Time to onset	20 hrs post C1D15	10.8 hrs	NR	1 day post C1D1 & C1D15	C1D1	NR
Median duration	48hrs	2.2 days	NR	2 days	2 days	NR

NR, not reported

Figure 1. Nursing interventions for cytokine release syndrome (CRS) during bispecific antibody treatment



Don't think twice, do an ICE...score.

McKeague M[1]
[1]*Royal Brisbane & Women's Hospital, Herston, Australia*

Introduction: Immune effector cell-associated neurotoxicity syndrome (ICANS) is a potentially serious complication of chimeric antigen receptor t-cell (CAR-T) therapy, effecting between 20-60% of all patients (Hayden et al. 2022). Adult patients undergoing CAR-T cell therapy are assessed for ICANS twice daily through using the 10-point immune effector cell encephalopathy (ICE) scoring tool (Lee et al. 2019). Urgent medical assessment is required for any drop in ICE score. Certain risk factors identifiable prior to CAR-T cell therapy, can determine patients predicted risk of developing ICANS, including cytokine release syndrome (CRS) following CAR-T cell infusion. Nurses play a key role in the early identification of ICANS in these patients as the nature of bed-side nursing allows them to identify subtle neurological changes.

Background: This case study will discuss a 62-year-old patient who underwent CD19 CAR-T cell therapy (axi-cel) as treatment for their relapsed refractory DLBCL and the subsequent development of ICANS.

This patient developed Grade 2 CRS on Day+1, with febrile episodes and occasional occurrences of hypotension. This was treated with intravenous (IV) fluids, IV antibiotics and tocilizumab (TCZ). On Day+3 following CAR-T cell infusion this patient had an ICE score of 9/10, attributed to worsening handwriting. On Day+5, a marked decrease in the ICE score from the previous day indicated a significant deterioration in the clinical status of the patient prompting nursing staff to activate a medical emergency. The patient was transferred to the intensive care unit (ICU) with Grade 3 ICANS. The clinical condition of the patient improved over the following 24 hours, who returned to the ward on Day+6 and progressively made a full recovery.

Conclusion: As utilisation of CAR-T cell therapy expands, nurses at the bedside will see more cases of ICANS. Optimising the nursing assessment and early management of these patients could lead to early identification of ICANS and therefore better outcomes.

Reference List:
Hayden, PJ., Roddie, C., Bader, P., Basak, GW., Bonig, H., Bonini, C., Chabannon, C., Ciceri, F., Corbacioglu, S., Ellard, R., Sanchez-Guijo, F., Jäger, U., Hildebrandt, M., Hudecek, M., Kersten, MJ., Köhl, U., Kuball, J., Mielke, S., Mohty, M., Murray, J., Nagler, A., Rees, J., Rioufol, C., Saccardi, R., Snowden, JA., Styczynski, J., Subklewe, M., Thieblemont, C., Topp, M., Ispizua, ÁU., Chen, D., Vrhovac, R., Gribben, JG., Kröger, N., Einsele, H., & Yakoub-Agha, I. (2022). Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). *Annals of Oncology*, vol. 33, no. 3, pp. 259–275.

Lee, DW., Santomasso BD., Locke FL., Ghobadi A., Turtle CJ., Brudno JN., Maus MV., Park JH., Mead E., Pavletic S., Go WY., Eldjerou L., Gardner RA., Frey N., Curran KJ., Peggs K., Pasquini M., DiPersio JF., van den Brink MRM., Komanduri KV., Grupp SA., Neelapu SS. (2019). ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant,* 25, 625-638. doi: 10.1016/j.bbmt.2018.12.758.

Transforming Survivorship Care: Patient informed follow up care after Allogeneic Stem Cell Trans-plantation

Cirone B[1], Klarica D[1], Spencer A[1,2], Wright T[1,3]
[1]Alfred Health, Melbourne , Australia, [2]Alfred Health-Monash University, Melbourne, Australia, [3]LaTrobe Regional Hospital, Traralgon, Australia

Aim: To examine the patient experience of attending a Long Term Follow Up (LTFU) clinic for individuals after allogeneic stem cell transplantation (alloSCT) and identify opportunities for consumer informed quality improvement.

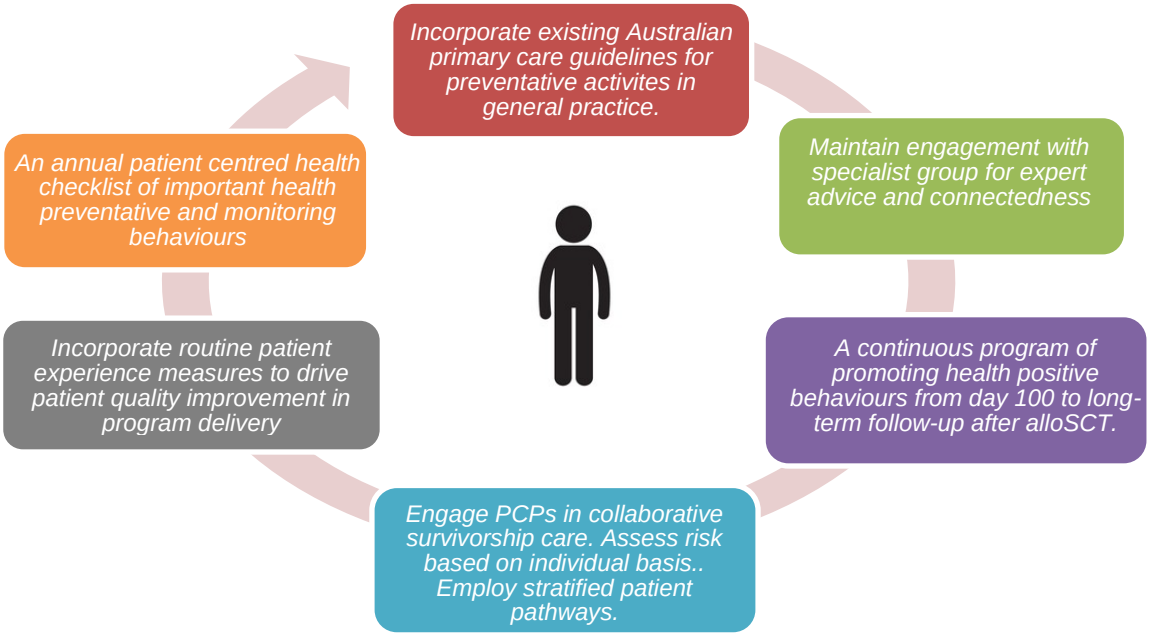
Method: Consecutive individuals attending the LTFU clinic surviving at least 2 years after alloSCT were enrolled in an interview based qualitative study. Interview questions are provided in Box 1 and were conducted by three experienced long-term follow up practitioners. Following each clinic, interviewers met and analysed responses, agreed on themes, and informative patient responses were identified. **Results:**

Box 1: Interview questions

1. Tell me about your experience of the late effects clinic, care plan and pre-clinic investigations
2. How does your attendance at the LTFU clinic relate to the care your receive from your primary care physician (PCP)
3. Do you have any recommendations for optimal focus of the LTFU clinic

Between January and November 2022 88 interviews were conducted. Forty three percent of participants were female; the median age at interview was 54 years and for most the primary disease was acute leukaemia 55 (63%). Fifty two (59%) of participants had a diagnosis of chronic graft versus host disease (cGvHD). The majority of patients 81 (92%) reported a positive response to attending the LTFU clinic, maintaining engagement with the specialist team was a recurring theme. A high number of participants 69 (78.4%) reported extensive pre clinic investigations were a burden and often replicated across health care visits including with PCP. Most participants 75 (88%) had a PCP and described confidence in their PCP providing non-specialist care. The most common response, for the optimal focus of the clinic was an accessible optimal health checklist, replacing the detailed care plan following transplant, not just restricted to the post 2-year period. Figure 1 summarises the consumer informed proposed changes to the delivery of supportive care after transplantation.

Figure 1: Key recommendations to post transplant supportive model of care.



Conclusion: We describe the value of incorporating consumer feedback to achieve patient-centred, safe and clinically relevant survivorship care following alloSCT.

Creation and implementation of a Haematopoietic Progenitor Cell (HPC) infusion policy, educational resources and competency assessment for nurses

Bubner K[1], Fergus A[2], Shooter E[3]
[1]CALHN Royal Adelaide Hospital , Adelaide, Australia, [2]CALHN Royal Adelaide Hospital, Adelaide, Australia, [3]CALHN Royal Adelaide Hospital, The Queen Elizabeth Hospital , Adelaide, Australia

Aim: No formal organisational policy or learning pathway has existed in Central Adelaide Local Health Network (CALHN) for the acquisition of theoretical knowledge and clinical skills in the administration of haemopoietic progenitor cell infusions. The aim of this nurse-led project was to scope and develop a formal organisational wide procedure, learning pathway and assessment tool that fosters the development of confident, skilled and knowledgeable clinicians within the Cancer program, enabling them to provide safe, evidence-based care to patients.

Method: Evaluation of various Bone Marrow Transplant Units across Australia was undertaken to understand current processes across other units. Many policies were available for the infusion of HPC product, however limited information was available for nursing specific training requirements. Using a co-design iterative process that included nursing, medical, apheresis and cellular therapy staff the organisational policy for all staff involved in HPC infusions was developed followed by a theoretical HPC Infusion workbook for nursing. A competency assessment tool was developed based on current clinical education tools and informed by organisational policy.

Results: The organisational wide policy and multi-modality learning package, designed to align with the National and International standards in haematopoietic cell therapy product collection, processing and administration were published in April 2023. The learning tools were piloted in the Allogeneic Bone Marrow Transplant Unit at RAH where testing continues.

Conclusion: Early feedback from nursing staff is favourable. Planning has commenced with the eLearning development team to translate the learning package onto the CALHN online learning platform. Additionally, a nursing instructional video demonstrating the administration of HPCs with the inclusion of the consumer HPC transplant experience is in production.

Implementation of a new extracorporeal photopheresis (ECP) service at the Royal Brisbane & Women's Hospitalnur

Mudie K[1]
[1]*Royal Brisbane & Women's Hospital, Herston, Australia*

Introduction: Extracorporeal photopheresis (ECP) is a leukopheresis based immunomodulatory therapy indicated for treatment of advanced stage erythrodermic cutaneous T-cell lymphoma (CTCL) and steroid refractory chronic graft-versus-host-disease (cGVHD).

CTCL is a rare lymphomatous disorder with limited curative treatment options for late-stage disease. Therapy focusses on inducing long-term remissions or palliative symptom control.

cGVHD occurs in 24-44% of allogeneic haematopoietic stem cell transplants in Australia (ABMTRR, 2020) typically requiring intensive long-term immunosuppression including prolonged steroids and calcineurin inhibitors with the potential for significant treatment-related toxicities.

Prior to 2022, ECP was not available in Queensland due to lack of government funding via the Medicare Benefits Schedule. Queensland patients were required to travel interstate for access, incurring significant financial expense and social disruption.

New service implementation: This presentation will detail the steps involved in introducing this new service. It involved multiple key stakeholders; development of new ECP treatment protocols and standard operating procedures; introduction of new patient referral and evaluation pathways; purchasing of ECP equipment; staff training; and ensuring that all requisite accreditation standards were met. Challenges included predicting potential patient numbers leading to difficulties in projecting resources required to successfully implement and maintain this new service.

A systematic and inclusive approach ultimately led to the successful rollout of the first ECP service in Queensland in September 2022.

Conclusion: ECP is a treatment modality that can improve the quality of life of refractory CTCL patients and is a potential curative treatment option for cGVHD patients.

The introduction and implementation of this ECP service provides improved accessibility and treatment options for Queensland patients. Ongoing evaluation of our service will monitor for efficient and cost-effective delivery, and patient outcomes.

CD3 cell dose in autologous haematopoietic stem cell transplant as a predictor of engraftment and of overall survival in patients with myeloma/lymphoma.

Charumbira E[1]

[1]SA Pathology - Royal Adelaide Hospital, Adelaide, Australia

Background and objective: It has been demonstrated in past studies that *allogeneic* transplants containing higher quantities of CD3 cells improve engraftment kinetics when compared to those with lower quantities Castillo *et al* (2016). The mechanism thought to be responsible for this association is that the CD3 cells in the graft provides lymphokines needed for stem cell differentiation and proliferation in the marrow niche. This study's aim was to explore this association between the number of infused CD3 (CD3+*i*) cells and engraftment, also survival, in *autologous* transplants in haematological malignancies.

Patients and method: Fifty-two (aged 18 to 75 years old) previously cryopreserved samples from myeloma and lymphoma patients who underwent autologous hematopoietic stem cell transplantation (HSCT) between 2014 and 2015 at the local hospital were thawed and analysed for CD3 cells. The CD3 cell numbers in the thawed samples were determined by flow cytometry for each patient and the doses compared against engraftment data provided by the hospital. The other inclusion criterion was that the stem cell transplant needed to have had a minimum CD34 dosage of 2.0×10^6/kg as this is widely considered to be the minimum dose required for successful engraftment. The instrument used for cell enumeration was the FACS Canto 1 and analysed using the Diva software.

Results: There was no statistically significant association between absolute lymphocyte count (ALC) and CD3+*i* at both days +15 and +30 post-HSCT.

Conclusion: The study findings were that there was no significant relationship between CD3 cell dose in autologous transplant bags and rate of engraftment from this cohort of patients. There was also no association between CD3 cell doses and survival both at 1-year and 2-years.

A network approach to implementing a laboratory aseptic technique competency

Kabani K[1], Leonard V[1], Palladinetti P[1]
[1]*Agency for Clinical Innovation, St Leonards, Australia*

Aseptic processing is challenging to validate as it involves trying to demonstrate there has been no breach of sterility. Although blood and marrow processing incorporates microbial screening of every product, sterility testing cannot be performed, and the products cannot be terminally sterilised. Environmental monitoring, under current accreditation frameworks such as NPAAC also does not mandate this to be performed for each individual product. This project was designed to assess the aseptic technique of processing scientists across a network of laboratories.

A validation plan was written detailing the use of Tryptone Soy Broth (TSB) as a substitute for a patient product. TSB supports the growth of a wide variety of microorganisms, especially common aerobic and facultative anaerobic bacteria. Because of its capacity for growth promotion, this formulation has been adopted by The United States Pharmacopeia (USP) and the European Pharmacopeia (EP) as a preferred sterility test medium.

The procedure aimed to replicate processing of HPC products which includes performing the range of connections and sampling techniques in a closed system. A minor difference, requiring an initial transfer of TSB from a bottle to a transfer bag was introduced this year. Processing included sterile welding or transfer via a spike into a transfer bag, centrifugation, addition of a simulated cryo-mixture and dispensing into freezing bags.

Sterility testing of the freezing bags was performed by a TGA licenced facility including a positive control and a statis test as a positive control. Details of the consumables and results were recorded on a standardised worksheet.

Initial results demonstrated a high level of aseptic technique and reproducible results across multiple laboratories. Results for the procedure that includes the minor modification will be analysed and presented. This competency procedure will be used annually to demonstrate continued competency to aseptically process HPC products to the highest international standard.

Building capacity to provide intensive haematology care in a regional setting

Ashby M[1,2], Wickramarachchi S[2], Jewell J[2], Wright T[1,2]
[1]*Alfred Health, Melbourne, Australia,* [2]*Latrobe Regional Hospital, Traralgon, Australia*

Aim: To describe the development of a myeloid program in a regional setting identifying clinical and system requirements through a patient case series.

Method: Supported by TrialHub*, Latrobe Regional Hospital (LRH) and Alfred Health (AH) established a haematology fellow program building a partnership to deliver complex care in Gippsland, including access to clinical trials for myeloid, myeloma and lymphoma programs. A case series of consecutive individuals living in Gippsland and diagnosed with Acute myeloid leukaemia (AML) or high-risk myelodysplastic syndrome (MDS) that were suitable for hypomethylating agents up front was collected. Patient demographics and clinical diagnosis are summarised. Details of optimal disease diagnostics and treatment, their location and omission are provided to highlight access barriers in regional settings.

Result: Six patients were identified between August 2022 and June 2023. All patients had BMAT completed with next generation sequencing (NGS) at AH. Table one summaries their demographics and clinical details, treatment and supportive care delivered between AH and LRH and clinical outcomes. Cut off for follow up was 1/6/23.

Table 1

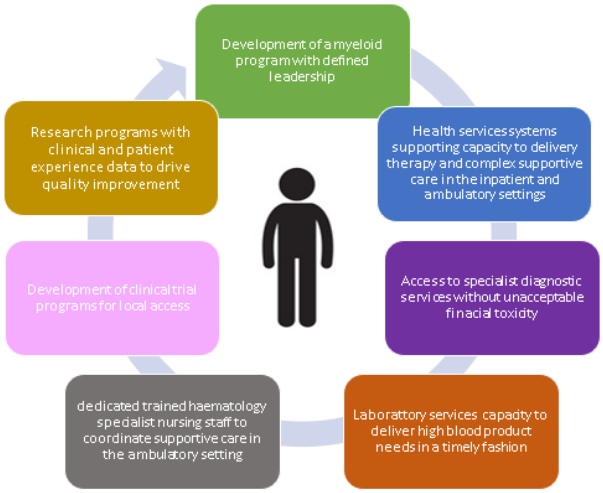
Case	Gender	Age	Diagnosis	ECOG	Other notes	Treatment		Supportive care				Outcome
						M	R	M		R		
								RBC	P	RBC	P	
1	M	80	Therapy related AML	1	Prev bowel Ca Presented in DIC	AZ C1	AZ C2-6	8	0	0	X0	Alive CR
2	F	76	sAML	2	Ongoing bone marrow aplasia post C1	V/AZ C1	nil	22	23	29	12	Died 168 days from diagnosis
3	M	68	MDS IB/AML	1		INQ C1	INQ C2-6	3	0	3	0	Alive CR
4	M	80	AML	1		V/AZ C1	V/AZ C2-6	10	1	2	0	Alive CR
5	M	76	AML	1	Commenced on M25 clinical trial	V/LDAC C1	V/LDAC C2-4	4	0	2	0	Alive CR
6	M	19	MDS IB/AML	0	Planned for transplant at metro		INQ C1-4	3	0	9	2	Alive CR

M=metro, R=regional, RBC=red blood cells, P=platelets, LDAC=Low dose cytarabine, INQ=INQOVI, V=venetoclax, AZ=azacitidine, CR=complete remission

Conclusion: The development of a safe and effective myeloid program in the regional setting requires attention to important diagnostic, treatment and supportive care elements. Through our metro – regional partnership key missing components are being established through strong mentorship focussed on sustainability.

Figure 1 Summarises the requirements for a myeloid program, established through patient data to enable the delivery of therapy for appropriate individuals in the regional setting.

Figure 1



Improving treatment selection of acute myeloid leukaemia (AML) patients over the age of 70

Ashby M[1], Chua C[1], Tiong I[1], Teh T[1], Fleming S[1]
[1]***Alfred Health, Melbourne, Australia***

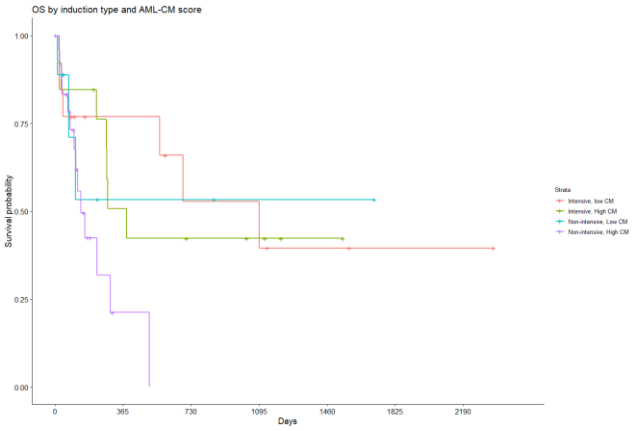
Aim: With increasing access of treatment for elderly AML patients, there is an increased burden of toxicity and hospital resources for frequently poor outcomes. We audited our AML patients >70 for outcomes in an attempt to improve patient selection for treatment and applied the AML composite model[1] (AML-CM) for comorbidity assessment.

Method: Retrospective review of hospital records of patients diagnosed with AML age ≥70 years from January 2016 – January 2023 and managed as standard of care as first line of therapy at Alfred Health, Melbourne, Victoria. Descriptive statistics were used and Kaplan-Meier estimate for survival.

Results:

Table 1 – Baseline Demographics	**Intensive (n=26)**	**Non-intensive (n=34)**
Age, median (range)	72 (70-78)	78 (70-91)
ELN criteria		
Not available	0	2 (6%)
Favourable	10 (38%)	4 (12%)
Intermediate	8 (31%)	8 (24%)
Adverse	8 (31%)	20 (58%)
Molecular		
FLT3	5/25 (1 NA)	5/28 (6 NA)
NPM1	8/25 (1 NA)	5/28 (6 NA)
TP53	4/22 (4 NA)	3/28 (6 NA)
Primary AML	22 (85%)	17 (50%)
Secondary AML	5 (15%)	13 (38%)
Therapy related	0	4 (12%)
Therapy		
7+3	12	
5+2	13	
FLAG-AMSA	1	
STIMULUS		5
LDAC		1
LDAC/VEN		6
AZA		3
VEN/AZA		19
AML-CM median (range)	6.5 (3-11)	8 (3-14)

Table 2 – Outcomes	**Intensive (n=26)**	**Non-intensive (n=34)**
Response rate after C1		
CR/Cri	14 (54%)	12 (35%)
Not CR/CRi (SD, MLFS, PR)	4 (15.3%)	9 (26%)
Progressive disease		
Not assessed	4 (15.3%)	4 (12%)
	4 (15.3%)	9 (26%)
OS, median (range) months	22.55 (0.43-77.36)	5.23 (0.43-56.32)
AML-CM <7.5	22.55 (0.43-77.36)	3.62 (0.46-56.32)
AML-CM >7.5	9.27 (0.72-50.79)	5.23 0.13-16.64)
Number of admissions, median (range)	3 (1-10)	2 (0-27)
Total LOS days median (range)	64 (14-137)	38 (0-100)



Conclusion: Amongst older adults receiving therapy for AML the survival outcome trended towards superiority for patients receiving remission induction chemotherapy (2-year OS of 47% vs 21%, p=0.054), however 2-year survival outcomes were similar amongst patients with a low AML-CM (6 or less) irrespective of therapy received (53% in both groups) while they were significantly improved within the group with a higher AML-CM (over 6) receiving intensive chemotherapy (2-year OS of 42.3% vs 0%, p=0.037), likely reflecting factors outside the AML-CM driving therapy choice in this group. Given the similarity in both intensive and non-intensive therapy both appear reasonable options for the treatment of fit older adults, however further larger prospective studies are warranted to confirm this. Further data on outcome and toxicity will be presented at the conference.

1. Sorror ML, Storer BE, Fathi AT, et al. Development and Validation of a Novel Acute Myeloid Leukemia–Composite Model to Estimate Risks of Mortality. JAMA Oncology 2017;3(12):1675-1682. DOI: 10.1001/jamaoncol.2017.2714.

Venetoclax based therapies as a bridge for allogeneic haematopoietic stem cell transplant for acute myeloid leukaemia (AML) and high-risk myelodysplastic syndrome (MDS): is it necessary and what is the goal?

Ashby M[1], Chua C[1], Fleming S[1]
[1]*Alfred Health, Melbourne, Australia*

Aim: For patients requiring allogeneic haematopoietic stem cell transplant (HSCT) for AML/MDS, maximising disease response is optimal. Venetoclax (VEN) based therapy allows low and high intensity combinations to be used to maximise disease response pre-HSCT whilst minimising toxicity. At our institutions, we use VEN combinations as a bridge to HSCT for select patients that either have MRD positivity or sub-optimal disease response pre-HSCT. We aimed to retrospectively investigate the safety, time to transplant and effectiveness of VEN based combinations in bridging patients to HSCT

Method: Retrospective review of patients treated with VEN based combinations from 2018-2022 immediately prior to allogeneic haematopoietic stem cell transplant for MDS (>10% blasts) and AML at Alfred Hospital and Northern Hospital, Melbourne, Australia. Patients were identified by pharmacy dispensing records and data collected from electronic medical record. Descriptive statistics were used.

Results:

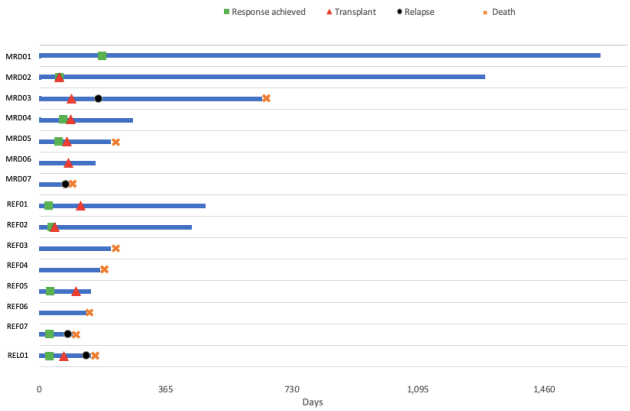


Table 1 - Baseline demographics		
Total		15
Male sex, n (%)		12 (80)
Age, median (range)		61 (35-73)
Diagnosis, n (%)	AML	12 (80)
	T-MN (AML)	1 (7)
	MDS-EB2	2 (13)
ELN 2022 risk category, n (%)	Favourable	3 (20)
	Intermediate	2 (13)
	Adverse	10 (67)
Treatment group, n (%)	MRD	7 (47)
	Refractory	7 (47)
	Relapse	1 (7)
Treatment, n (%)	Ven/LDAC	9 (60)
	Ven/Aza	4 (27)
	FLAVIDA	2 (13)
Number of cycles, median (range)		2 (1-4)
Molecular mutations at time of treatment, n (%)	NPM1	3 (20)
	IDH2	5 (33)
	NPM1 and IDH2	1 (7)
	FLT3	2 (13)
Prior treatments, n (%)	Intensive	12 (80)
	CPX351	2 (13)
	Azacitidine + magrolimab/placebo	1 (7)

Conclusion: VEN combinations have minimal treatment related toxicity pre-HSCT. We demonstrate successful use in both MRD eradication and inducing remission in relapsed and refractory AML patients. Further questions remain including the optimal goal of pre-transplant treatment and the impact of transplant delay and toxicity of these treatments.

Validation and Implementation of Cepheid Xpert® BCR-ABL Ultra p190 Assay for Diagnosis and Disease Monitoring in Acute Lymphoblastic Leukaemia (ALL).

Bradshaw G[1], De Silva W[1], Lin W[1], Le A[1], Koshy A[1], Markalanda D[1], Agarwal R[1]
[1]*Austin Health, Heidelberg, Australia*

Aim: Xpert® BCR-ABL is a quantitative closed-system test providing high sensitivity results (LoD 0.0065%) in ~2.5hrs, reducing time-consuming hands-on processes including RNA extraction, as compared to RT-qPCR. Our aim was to validate the Xpert® BCR-ABL p190 assay (Class 3 IVD) for routine use on diagnostic and monitoring blood and bone marrow (BM) samples for ALL.

Method: We have validated blood and BM (n=25) for diagnostic and MRD monitoring for the p190 (e1a2) transcript by GeneXpert®. For blood/BM samples with WCC >30x10^6/mL, 50µL input volume was used. For blood samples with WCC <30x10^6/mL, 4mL input volume was used. Range of detection of the assay is 0.0065% - 25%. 25 samples from known Ph+ ALL patients were tested by both Ipsogen RT-qPCR and Xpert® methods and concordance between results was assessed. INTROL BCR-ABL p190 control panel (MMQCI, USA) was tested in duplicate on Xpert® covering 5 levels of detection (0%, 0.02%, 0.1%, 1%, 10%) and our levels were compared with levels measured by MMQCI.

Results: 24 samples showed concordant results between real-time qPCR and Xpert®. Of these, 6 were diagnostic BM and 18 were monitoring samples (10 blood, 8 BM). One sample showed <10 BCR-ABL copies by RT-qPCR (above LOD >0.0069%) and No Detectable transcripts by Xpert®. As p190 results are not reported on IS, a direct comparison between the two assays is not possible, and hence clinical correlation and testing with both RT-qPCR and Xpert® will continue for cases with low level disease. MMQCI controls were all detected with high correlation between expected levels (R^2=0.982).

Conclusion: The Cepheid Xpert® BCR-ABL Ultra p190 test has been validated and deemed fit for use on diagnostic samples, as well as on monitoring blood/BM samples. This efficient and cost-effective assay will dramatically reduce testing turnaround times and free up staff for other important laboratory work. Ongoing correlation with RT-qPCR will increase study sample size and data will be updated at the time of the Blood conference.

Toxic myositis due to long term accumulation of rosuvastatin due to drug-drug interaction with enasidenib

Cashion C[1], Mason G[1], Parratt J[3,4], Stevenson W[1,2]
[1]Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, St Leonards , Australia, [2]Northern Blood Research Centre, Kolling Institute of Medical Research, University of Sydney, St Leonards, Australia, [3]Faculty of Medicine, University of Sydney, Sydney, Australia, [4]Department of Neurology, Royal North Shore Hospital, St Leonards, Australia

Abstract: Enasidenib is an oral IDH2 inhibitor which has been shown to improved event free survival in older patients with IDH2 positive relapsed/refractory acute myeloid leukaemia (AML). Its favourable side effect profile and oral administration makes it a promising option for patients for whom quality of life is paramount. Enasidenib has important drug interactions due to its inhibition of many CYP pathways, transporters and UGT1A1 as demonstrated by *in vitro* and pharmacokinetic studies. Polypharmacy, often with implicated medications, is common among the elderly population in whom enasidenib is currently in use.

We present a case of an 84-year-old woman with IDH2 positive AML who was treated with enasidenib while taking rosuvastatin. She presented with severe proximal muscle weakness with biochemical and MRI evidence of myositis after 2 years of concomitant therapy. This is the first report of a clinically significant interaction between enasidenib and statin therapy. This case highlights the importance of a thorough medication review and re-evaluation of the benefit of medications in this elderly population with limited life-expectancy to avoid adverse effects and optimise quality of life.

Untangling the clonal architecture in a case of acute myeloid leukaemia with multiple cytogenetically distinct clones

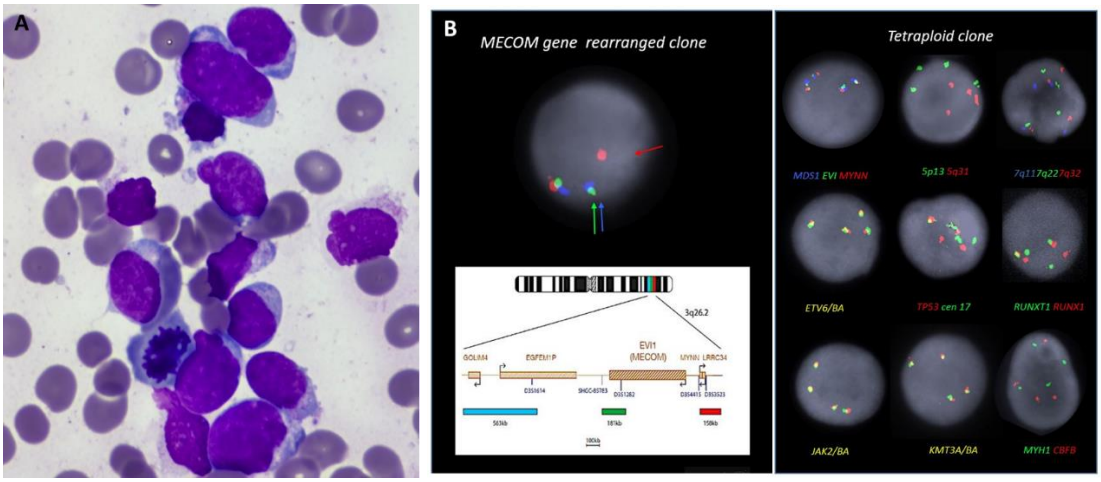
Cashman H[1], Xu K[1], Wilson A[1], Nacheva E[3], Baker R[2], Gupta R[1]

[1]Department of Haematology, University College London, , United Kingdom, [2]UCLH Specialist Integrated Haematology Malignancy Diagnostic Service, Health Services Laboratories, , United Kingdom, [3]HSL Analytical LLP, OncoGenomics, , United Kingdom

A 36 year old female presented with pancytopenia at 33 weeks of pregnancy in the context of COVID-19 infection. There was no history of pre-existing myelodysplasia or chemoradiotherapy. Bone marrow (BM) examination confirmed acute myeloid leukaemia (AML) with 82% myeloid blasts on BM aspirate smear (Figure 1A). Whole BM FISH identified two cytogenetically distinct non-overlapping sub-clones – one of which was tetraploid, the other marked by a *MECOM* rearrangement (Figure 1B). Whole BM NGS identified pathogenetic mutations in *BCOR* and *RUNX1*. Induction chemotherapy with FLA-IDA chemotherapy (fludarabine, cytarabine, idarubicin) was commenced six days following early delivery at 34+5/40. Complete morphological and cytogenetic remission was confirmed following cycle 2 of FLA-IDA. Interestingly, prior to planned allogeneic haemopoietic stem cell transplant (HSCT) a shoulder joint fluid aspirate performed to investigate a joint effusion confirmed a *MECOM* rearrangement in 6% of cells (23/380 cells) with no abnormalities on NGS. Venetoclax/azacitidine was administered as a bridge to myeloablative matched unrelated donor HSCT with morphological, flow cytometric and cytogenetic remission with 100% donor chimerism confirmed post HSCT.

AML arises through the stepwise accumulation of transforming mutations in haematopoietic stem and progenitor cells. Leukaemic cells exist in cellular hierarchies analogous to those seen in normal haematopoiesis, with the net result that in any AML, single cell analysis identifies multiple related leukaemic sub-clones, harbouring overlapping combinations of genetic mutations that define the bulk leukaemia. In the diagnostic laboratory, cytogenetic and molecular analysis of bulk bone marrow provides all the information required for rapid risk assessment and treatment of new cases of AML. The hetero-cellular nature of AML is rarely apparent in this setting - although it may become more obvious through treatment as dominant clones emerge. We were able to elucidate the likely clonal architecture of our patient's AML by monitoring her clinical progress using routine diagnostic assays.

Figure 1: Bone marrow investigations at diagnosis (https://doi.org/10.37946/bsh.img.87655)



- Bone marrow aspirate morphology at diagnosis, demonstrating 82% blasts without specific morphological features and B) FISH at diagnosis demonstrating two separate clones; a tetraploid clone and a *MECOM* rearranged clone

Infection and related mortality from Venetoclax therapy in patients with relapsed or refractory acute myeloid leukaemia: a single centre retrospective study2

Chandran D[1], Viiala N[2], Watson A[2], Dunlop L[2], Motum P[2], Sungala N[2], Range H[2], Getta B[2]
[1]*Westmead Hospital, Sydney, Australia,* [2]*Liverpool Hospital, Sydney, Australia*

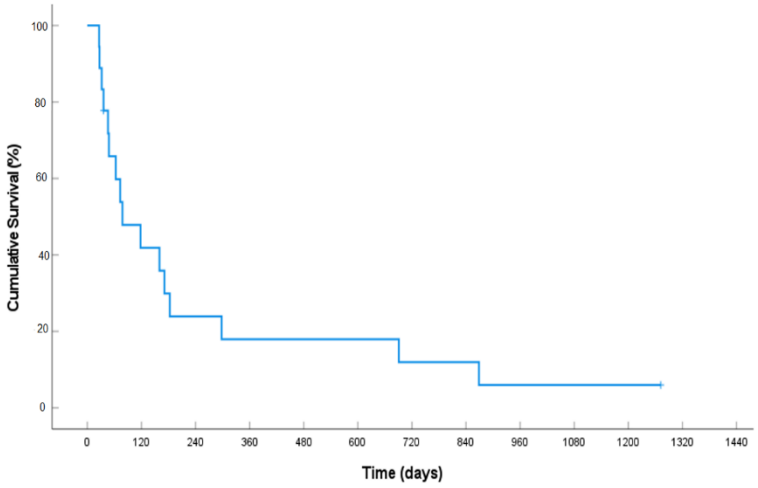
Aim: To quantify infective complications, disease response and overall survival in patients receiving Venetoclax therapy for relapsed/refractory acute myeloid leukaemia (R/R AML).

Method: This was a retrospective study investigating infective complications in 18 patients with R/R AML who received Venetoclax based therapy between 2018-2021 in a single centre using electronic medical records. Venetoclax was sourced through compassionate access limited to subjects with R/R AML and ECOG 2 or less with no alterative therapy options. Incidence of infections, febrile neutropenia, disease response and mortality were recorded for cycle 1 and separately for additional cycles.

Results: 4 patients had de novo and 14 secondary AML with median number of prior therapy lines being 2 (1-4). 9 (50%) had adverse genetic risk as per ELN 2017 stratification. 61% suffered infection in cycle 1, including 2 cases of PJP, 5 bacteraemias and 2 Aspergillus invasive fungal infections (IFI). Cycle 1 infectious mortality rate of 33%. 57% who received more than 1 cycle died within 6 months, all from sepsis. ORR was 29% among 14 patients with documented responses. The median OS was 70 days (95% CI 5-150 days). 3 (17%) survived more than 12 months, 3 underwent allograft, 1 remains alive 3 years post allograft and 1 lost to follow up.

Table 1. Demographics and Treatment Characteristics	
Median age, years (range)	67 (53-81)
Sex	13 Male / 5 Female
Venetoclax combination (N) Monotherapy LDAC Azacitidine	 3 11 4
Cycles of Venetoclax 1 cycle >1 cycle	 11 7

Figure 1: Overall Survival in Patients Receiving Venetoclax for R/R AML



Conclusion: High rates of infection and related mortality were observed in patients receiving Venetoclax for R/R AML from neutropenic sepsis. Of note, there were 2 cases of PJP and 2 IFIs suggesting anti-fungal and PJP prophylaxis should be strongly considered. The response rates after cycle 1 were modest with ORR 29% and median OS of 70 days.

Acute myeloid leukaemia with plasmacytoid dendritic cell-like phenotype – a case report with literature review

Chen S, Lacey C, Wong C
[1]*Australian Clinical Labs, Clayton, Australia*

Aim: Identification of an association between plasmacytoid dendritic cells (pDCs) and myeloid neoplasms can be challenging and may be underestimated due to rarity of the entities. An accurate diagnosis of plasmacytoid dendritic cell neoplasms and myeloid neoplasms with plasmacytoid dendric cell differentiation is important as patients may get benefits from anti-123 treatment for both conditions.

Method: The case review was conducted including medical history, presentations, clinical manifestations, and investigation results. A literature review was then performed regarding diagnostic criteria for myeloid neoplasms with pDC-like phenotype.

Results: A 76-year-old man with history of multiple myeloma 5 years post treatment presented with progressive pancytopenia. The peripheral blood film showed pancytopenia with 17% circulating blasts. The flow cytometry results on the peripheral blood demonstrated the presence of myeloblasts with pDC-like phenotype: CD34+(bright), CD117+(bright), CD13+(dim), CD11b+ (partial), CD38+, CD123+ (bright), CD2+ (partial), CD4+, CD7+ and CD56+. They are negative for cytMPO, cytCD3, sCD3, CD19, cytCD79a, CD10, CD14, CD15 and CD64. A bone marrow biopsy was done with a dry tap aspirate and blast infiltration in the bone marrow trephine. The features on the immunohistochemistry stains were consistent with acute myeloid leukaemia.

A literature search for plasmacytoid dendritic neoplasms and myeloid neoplasms with pDC-like phenotype or pDC-differentiation was reviewed. The diagnosis of acute myeloid leukaemia with pDC-like phenotype for this case was made. On the background of myeloma treated with VCD (6 years prior) and Melphalan autograft (5 years prior) together with the absence of skin lesions or other extramedullary disease on radiological survey, the final WHO diagnosis of therapy-related acute myeloid leukaemia was achieved.

Conclusion: Diagnosis of myeloid neoplasms with pDC-like phenotype or pDC-differentiation is increasingly recognised and is becoming important for patient's management.

Clinical utility of chromosomal microarray analysis in patients with Acute Myeloid Leukaemia, Myelodysplastic Neoplasm or Myelofibrosis where conventional cytogenetic results are uninformative.

D'Achille P[1], Shi E[1], Dun K[1], Ninkovic S[1,2]
[1]*Victorian Cancer Cytogenetics Service, St. Vincent's Hospital Melbourne, Fitzroy, Australia,*
[2]*Department of Haematology, St. Vincent's Hospital Melbourne, Fitzroy, Australia*

Aim: Conventional cytogenetics (CC) are an integral component of the diagnostic workup in acute myeloid leukaemia (AML), myelodysplastic neoplasm (MDS) and myelofibrosis (MF); confirming a diagnosis and allowing for risk stratification while guiding treatment decisions. This study was designed to assess the suitability of chromosomal microarray analysis (CMA) as a supplementary test in pts with AML, MDS or MF where due to either no/low mitotic index or poor chromosome morphology, CC failed.

Method: Between September 2020 and January 2023, CMA (Illumina Infinium global screening array V3.0) was performed on 55 Victorian pts, AML (n=24), MDS (n=14) and MF (n=17) who at diagnosis had an uninformative karyotype. The detected CMA variants were classified and reported according to genome build GRCh37 and consensus recommendations from the American College of Medical Genetics and Genomics and the Cancer Genomics Consortium[1]. Targeted fluorescence in situ hybridisation (FISH) testing was performed as required.

Results: CMA testing was successful in all 55 cases with acquired clonal genomic imbalance detected in 100% of cases. Tier 1 variants with strong clinical significance were detected in 24/55 (43.6%) cases, likely leading to change in treatment strategies, 23/55 (41.8%) of the cases had no Tier 1 but at least one Tier 2 variant with some clinical significance, while 8/55 (14.6%) had Tier 3 markers of clonality detected. In 11/55 (20%) cases, FISH was required to confirm low level variants detected by CMA or balanced structural chromosomal rearrangements which cannot be confidently identified by CMA alone. Additionally, CMA provided information on cytogenetically cryptic changes including copy neutral loss of heterozygosity (CN-LOH) of 17p (TP53) in a patient with AML

Conclusion: In AML, MDS and MF, CMA is a suitable alternative testing method when an informative result cannot be obtained using conventional CC methods and should be routinely offered as part of a comprehensive diagnostic genomics service.

References:

- Mikhail FM, Biegel JA, Cooley LD, et al. Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). Genet Med. 2019;21(9):1903-1916. doi:10.1038/s41436-019-0545-7.

Detection of Blinatumomab-mediated T cell synapse formation in B-ALL patients using flow cytometry.

Davis J[1,2], Peng H[1,2], Ludford-Menting M[1,2], Fleming S[3], Koldej R[1,2], Ritchie D[1,2,4]
[1]ACRF Translational Research Laboratory, The Royal Melbourne Hospital, Parkville, Australia, [2]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne , Parkville, Australia, [3]Clinical Haematology, Alfred Health, Melbourne, Australia, [4]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Parkville, Australia

Aim: Blinatumomab (blin) is a CD3/CD19 bispecific T-cell engager that has demonstrated notable efficiency in achieving minimal residual disease remission in relapsed/refractory B-cell acute lymphoblastic leukaemia (B-ALL) patients. Blin responses are more effective in patients with a higher baseline percentage of CD8+ T-cells and in patients with minimal residual disease. However, it is unknown whether T cell function has an impact on the therapeutic efficacy of blin. In this study, we examined the ability of HD and B-ALL patient-derived T-cells to form immunological synapses, and the memory phenotypes of responding T-cells.

Method: T cells isolated from PBMC of HD (n=10) and treatment-naïve B-ALL patients (n=7) were stained with CD45RA and CCR7 monoclonal antibodies prior to co-incubating with Raji tumour cells labelled with CD20, in a 1 hr synapse assay in the presence of blin (10 ng/ml). Conjugates were identified by flow cytometry as CD4+ and CD8+ T cells that co-expressed CD20. The phenotype of T cell memory subsets was defined as naïve (CCR7+CD45RA+), central memory (CCR7+CD45RA-), effector memory (CCR7-CD45RA-) and TEMRA (CCR7-CD45RA+). Data was analysed using the Wilcoxin-matched pairs T test or the Mann-Whitney unpaired T test.

Results: We developed a novel method using flow cytometry and monoclonal antibody staining to identify synapse formation between T cells and tumour target cells. Synapse formation increased in the presence of blin, and B-ALL patient T cells formed significantly more blin-mediated synapses than HD T cells. The expression of CD45RA on untreated T cells was positively correlated with blin-mediated synapse formation.

Conclusion: The synapse flow cytometry assay demonstrated that CD45RA+ T cells from B-ALL patients formed blin-mediated conjugates with Raji target cells. Future directions should examine the correlation of synapse formation with clinical outcomes in patients, biomarker identification, and functional T cell assays including cytotoxicity, degranulation and cytokine release.

Real-world outcomes in NPM1-mutated acute myeloid leukaemia and utilisation of measurable residual disease

Goh A[1,2], Weber N[1,2]
[1]*Royal Brisbane and Women's Hospital, Herston, Australia,* [2]*The University of Queensland, St Lucia, Australia*

Aim: NPM1-mutated (NPM1m) AML displays diverse clinical and genomic features with co-mutation status and measurable residual disease (MRD) most commonly used to identify patients who may benefit from allotransplantation. This study aimed to evaluate local outcomes in NPM1m AML, including impact of *FLT3*-ITD co-mutation status and post-remission treatment. We sought to describe utilisation of MRD in current practice and to validate published MRD thresholds for disease relapse and survival.

Method: All newly diagnosed NPM1m AML cases at the Royal Brisbane and Women's Hospital from 1 November 2015–30 June 2022 were included. Data was collected using electronic medical records including demographics, treatment details, relapse and survival. MRD data was collected and analysed according to changes with treatment, timepoints of evaluation and outcomes. Statistical analyses were performed using IBM SPSS.

Results: Forty-nine patients with NPM1m AML were identified. The median age was 60 years and 41% patients were male (Table 1). Forty-two patients underwent intensive chemotherapy and were included in subsequent analyses. Induction failure was more common in *FLT3*-ITD patients (15% vs 0%, p=0.048). Thirteen patients underwent allograft in CR1, of whom the majority (85%) had *FLT3*-ITD. The median LFS and OS was 46 and 52 months respectively (Figure 1), with comparable outcomes seen in *FLT3*-ITD patients (Figure 2). *FLT3*-ITD patients who did not undergo CR1 allograft had poor survival (n=5, median OS 12 mo). Of the eight *FLT3*-ITD negative patients who relapsed, three (37.5%) remain alive in CR2 after salvage allograft. Correlation of outcomes with MRD is ongoing.

Conclusion: Real-world outcomes for NPM1m AML remain disappointing despite the increasing sophistication of molecular prognostic tools. Survival outcomes in our dataset did not appear to be affected by *FLT3*-ITD status, likely reflecting a treatment bias towards CR1 allograft for mutated patients.

Table 1. Baseline characteristics of the NPM1-mutated AML patient cohort.

Table 1. Baseline Patient Characteristics	
Age	
Median	60 years
Range	16 – 81 years
Sex	
Male	20 (41%)
Female	29 (59%)
FLT3-ITD status	
FLT3-ITD	22 (45%)
FLT3-wt	27 (55%)
Other Co-mutations	
FLT3-TKD	8 (16%)
DNMT3A	7 (14%)
IDH 1/2	9 (18%)
Karyotype	
Normal	45 (92%)
Abnormal	4 (8%)
Intensive Therapy	
Yes	42 (86%)
No	7 (14%)

Genomic screening in advanced haematological cancers: Lessons from the first 63 cases in the Australian Molecular Screening and Therapeutics in Leukaemia and Lymphoma (MoST-LLy) program

Grove C[1], Brown A, Dobbins J, Amor G, Diamond P, Harrup R, Grady J, Lin F, Tan X, Blunt D, D'Andrea R, Ross D, Scott H, Lane S
[1]Sir Charles Gairdner Hospital, PathWest, Linear, Perth, Australia, [2]SA Pathology, Adelaide , Australia, [3]QIMR, Brisbane, Australia, [4]Leukaemia Foundation, Adelaide, Australia, [5]Royal Hobart Hospital, Hobart, Australia

Aim: Patients with relapsed or refractory high-grade lymphoma or leukaemia have an extremely poor prognosis. Recent advances in genomics have identified distinct, molecularly-defined, biological subgroups in these diseases, revealing some targetable vulnerabilities. Nevertheless, widespread access to genomic profiling and clinical trials of novel targeted therapies remains challenging. MoST-LLy, an MRFF-funded, innovative platform trial from a nationwide collaboration, is designed to provide access to broad genomic profiling to identify targetable variants in patients with high-risk blood cancers and link relevant clinical trials to accelerate drug development and improve outcomes in this patient group.

Method: This ongoing national program aims to recruit 480 high-risk blood cancer patients. The program incorporates the 523-gene TSO 500 (Illumina) sequencing panel, reporting through a national haematology Molecular Tumour Board for identification of patients suitable for biomarker-directed clinical trials. Two investigator-initiated phase 2 trials associated with the program are currently recruiting (ACTRN12621000507886 and ACTRN12621001183875). Translational research programs are planned to identify biomarker correlates of response in exceptional responders.

Results: As of May 2023, molecular profiling has been completed on 63/80 consented patients (32 with high-grade lymphoma and 31 with high-risk leukaemia) recruited through sites in Brisbane, Adelaide, Perth and Hobart. Clinical trial recommendations were shortlisted by matching the actionable variants or molecular biomarkers against the TOPOGRAPH knowledgebase. Forty-eight of 63 patients (76%) have had at least one clinical trial recommendation, resulting in 19 people (30%) receiving further therapy on a biomarker-linked or histology-based trial. Genomic profiling has also led to therapy change outside of trial in some cases. Updated sequencing and outcome data will be presented at the meeting.

Conclusion: The MoST-LLy platform has demonstrated the feasibility of implementing a nationwide research infrastructure to systematically screen patients with refractory blood cancers using genomic profiling. This innovative paradigm allows efficient linkage of patients to biomarker-linked studies that would otherwise be inaccessible to the broader population.

Olverembatinib (HQP1351) overcomes ponatinib resistance in patients with heavily pretreated/refractory chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL)

Jabbour E[1], Koller P[2], Oehler V[3], Jamy O[4], Mukherjee S[5], Hunter A[6], Baer M[7], Beck J[8], Chen Z[9], Guo H[10], Fu L[11], Men L[9], Jiang L[11], Wang C[11], Wang H[9], He Z[12], Chen J[12], Yang D[9,10,11], Zhai Y[9,10,11], Kantarjian H[1]
[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, US, [2]City of Hope National Medical Center, Duarte, US, [3]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, US, [4]Division of Hematology and Oncology, Department of Medicine, University of Alabama at Birmingham, Birmingham, US, [5]Department of Hematology and Medical Oncology, Leukemia Program, Taussig Cancer Institute, Cleveland Clinic, Cleveland, US, [6]Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, US, [7]University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, US, [8]Highlands Oncology Group, Fayetteville, US, [9]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, CN, [10]Guangzhou Healthquest Pharma Co. Ltd., Guangzhou, CN, [11]Ascentage Pharma Group Inc., Rockville, US, [12]Ascentage Pharma Pty Ltd., Sydney, AU

Aim: This study reports safety, efficacy, and pharmacokinetic (PK) profiles of novel third-generation BCR-ABL1 tyrosine kinase inhibitor (TKI) olverembatinib in patients outside China with CML (all phases) and Ph^+ ALL, whose disease failed ponatinib treatment and ≥ 2 prior TKI treatments.

Method: Olverembatinib was administered orally on alternate days (QOD) in adults with CML or Ph^+ ALL. Enrolled patients had no fewer than 2 prior TKI failures, except for those whose disease harbored T315I mutation, for whom the number of previous TKIs was not limited. Study participants were randomized 3:3:2 to olverembatinib 30, 40, or 50 mg QOD in 28-day cycles.

Results: As of December 5, 2022, 46 patients were enrolled, including 36 with chronic phase (CP)-CML, and 10 with advanced Ph^+ ALL. The median (range) treatment duration was 4.8 (0.03-21.29) months. Twenty-one (70.0%) patients were pretreated with third-generation TKI ponatinib, including 17 (81.0%) with resistance and 4 (19.0%) with intolerance. Thirty-four (73.9%) patients experienced any-grade treatment-related adverse events (TRAEs; Table 1), incidences of which tended to be dose dependent. Common grade 3/4 hematologic TRAEs included thrombocytopenia (7/34; 20.6%), neutropenia (6/34; 17.6%), and decreased leukocyte counts (5/34; 14.7%). Of 23 efficacy-evaluable patients, 18 were evaluable for cytogenetic response, of whom 14 (77.8%) had complete cytogenetic response (CCyR) and 10/23 (43.5%) had major molecular responses (MMR). Olverembatinib was effective in patients with either T315I-mutant (87.5%, CCyR; 55.6%, MMR) or unmutated T315I (70.0%, CCyR; 35.7%, MMR), and its effectiveness was not compromised by prior use of ponatinib or allosteric STAMP inhibitor asciminib. PK analyses indicated an approximately dose-proportional increase in olverembatinib plasma exposure and comparable plasma exposure between Chinese and US CML populations.

Conclusion: Olverembatinib monotherapy is efficacious and well tolerated in patients with TKI-refractory CML and Ph^+ ALL, including those with ponatinib failure or T315I mutation. Internal study identifier: HQP1351-CU101; NCT04260022.

Table 1. Treatment-related adverse events (≥ 5% any grade), N = 34

Preferred Term, no. (%)	Any Grade	Grade 3-4	Serious
Thrombocytopenia	11 (32.4)	7 (20.6)	0
Blood creatine phosphokinase increased	9 (38.2)	5 (14.7)	2 (5.9)
Headache	8 (23.5)	0	0
Alanine aminotransferase increased	9 (26.5)	2 (5.9)	0
Neutropenia	6 (17.6)	6 (17.6)	1 (3.0)
Aspartate aminotransferase increased	8 (23.5)	2 (5.9)	0
Fatigue	8 (23.5)	0	0
Lipase increased	7 (20.6)	3 (8.8)	0
Myalgia	5 (14.7)	0	0
Nausea	6 (17.6)	0	0
Vomiting	4 (11.8)	0	0
Leukocytes decreased	5 (14.7)	5 (14.7)	0
Blood cholesterol increased	3 (8.8)	0	0
Rash	3 (8.8)	0	0

Skin hyperpigmentation	4 (11.8)	0	0

Adolescent/Young Adult B-ALL patients enrolled in the Australasian Leukaemia & Lymphoma Group ALL09 "SUBLIME" study have increased cytotoxic T-cells following exposure to blinatumomab.

Heatley S[1,2], McClure B[1,2], Schutz C[1], Eadie L[1,2], Page E[1,2], Rehn J[1,2], Yeung D[1,2,3,4,6], Greenwood M[3,5,6], White D[1,2,3,6]

[1]Precision Cancer Medicine Theme, South Australian Health and Medical Research Institute, Adelaide, Australia, [2]Faculty of Health & Medical Science, University of Adelaide, Adelaide, Australia, [3]Australasian Leukaemia & Lymphoma Group, Richmond , Australia, [4]Royal Adelaide Hospital, Adelaide, Australia, [5]Royal North Shore Hospital, Sydney, Australia, [6]These authors contributed equally, ,

Aim: Adolescents and young adults (AYA) with B-cell acute lymphoblastic leukaemia (B-ALL) are at higher risk of relapse compared with children, often due to more complex disease. The Australasian Leukaemia & Lymphoma Group (ALLG) ALL09 study substitutes chemotherapy with the Bi-specific T-cell Engager (BiTE) blinatumomab in consolidation in order to improve outcomes in newly diagnosed AYAs with B-ALL. This correlative study tracks changes in T-cell activation.

Method: Bone marrow (BM) samples were collected from 54 patients enrolled in the ALLG ALL09 study at diagnosis, day 33 (start blinatumomab) and day 79 when minimal residual disease is evaluated. BM mononuclear cells were assessed for T-cell activation markers (CD3, CD4, CD8, CD25, CD27, CD38, CD45RA, CD69, CD197 & HLA-DR) by flow cytometry. Data was analysed by FlowJo and statistical analyses by Kruskal-Wallis test with multiple comparisons was performed in GraphPad Prism, results are presented as the median and $p<0.05$ was considered significant.

Results: As expected, there was a significant increase in the proportion of T-lymphocytes (CD3) from diagnosis to day 33 (8% v 74%, $p<0.0001$) in response to chemotherapy; however, there was no significant difference between day 33 and day 79. A significant decrease in T-helper cells (CD3+/CD4+) (55% v 43%, $p<0.0001$) and increase in cytotoxic T-cells (CD3+/CD8+) was noted between day 33 and day 79 (38% v 47%, $p<0.0001$). There were no significant differences between diagnosis and day 33. Furthermore, activation markers HLA-DR & CD38 were significantly decreased from diagnosis to day 33 and increased at day 79 in both T-helper and cytotoxic T-cell subsets (Table 1).

Conclusion: This study has demonstrated that during standard chemotherapy the activation status of T-cells is suppressed; however, upon the addition of blinatumomab the level of activation increases back to pre-treatment levels. While there was no increase in the proportion of T-cells, after the addition of blinatumomab, the BM T-cells become cytotoxic enabling the potential elimination of leukaemic cells.

Table 1. T-cell Activation markers

	Diagnosis % median	Day 33 % median	Dx v D33 P value	Day 79 % median	D33 v D79 P Value
T-Helper					
HLA-DR+	4	0.6	0.0002	2.7	0.0001
CD38+	11.25	2.5	<0.0001	10.6	<0.0001
Cytotoxic					
HLA-DR+	5	0.99	<0.0001	5.8	<0.0001
CD38+	44	17	0.0032	52	<0.0001

Impact of age, prior therapies, and subsequent transplant on long-term outcomes of adults with relapsed or refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) treated with brexucabtagene autoleucel (brexu-cel) in ZUMA-3

Shah B[1], Cassaday R[2], Park J[3], Houot R[4], Oluwole O[5], Logan A[6], Boissel N[7], Leguay T[8], Bishop M[9], Topp M[10], O'Dwyer K[11], Baer M[12], Schiller G[13], Abedi M[14], Minnema M[15], Stiff P[16], **La Greca N[17]**, Zhou L[17], Hadjivassileva T[17], Damico Khalid R[17], Ghobadi A[18]
[1]Moffitt Cancer Center, Tampa, USA, [2]University of Washington & Fred Hutchinson Cancer Center, Seattle, USA, [3]Memorial Sloan Kettering Cancer Center, New York, USA, [4]CHU Rennes, Univ Rennes, Inserm & EFS, Rennes, France, [5]Vanderbilt University Cancer Center, Nashville, USA, [6]UCSF Medical Center, San Francisco, USA, [7]Hôpital Saint-Louis, Paris, France, [8]Service d'hématologie clinique et thérapie cellulaire Hopital du Haut-Leveque CHU de Bordeaux, Bordeaux, France, [9]The University of Chicago Medicine, Chicago, USA, [10]Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Würzburg, Germany, [11]Wilmot Cancer Institute of University of Rochester, Rochester, USA, [12]University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, USA, [13]David Geffen School of Medicine at UCLA, Los Angeles, USA, [14]University of California Davis Comprehensive Cancer Center , Sacramento, USA, [15]University Medical Center Utrecht, on behalf of HOVON/LLPC, Utrecht, The Netherlands, [16]Loyola University Chicago Stritch School of Medicine, Maywood, USA, [17]Kite, a Gilead Company, Santa Monica, USA, [18]Washington University School of Medicine, St Louis, USA

Aim: Brexu-cel is approved for R/R B-ALL in the US and Australia (≥18 years) and the EU (≥26 years). Here we report 3-year outcomes in ZUMA-3 by age, prior therapies, and subsequent allogeneic stem cell transplant (alloSCT).

Method: Patients (≥18 years) had R/R B-ALL and received one brexu-cel infusion (1×10^6 CAR T cells/kg). Primary endpoint was overall complete remission (CR)/CR with incomplete hematologic recovery (CRi) rate per independent review. Post hoc subgroup analyses were exploratory.

Results: As of July 23, 2022, median follow-up in Phase 2 (N=55) was 38.8 months (range, 32.7-44.6). CR/CRi rates (95% CI) were 67% (35-90) for patients <26 years (n=12) and 72% (56-85) for patients ≥26 years (n=43). Median (95% CI) OS was 28.6 months (0.6-not estimable [NE]) and 34.1 months (15.9-NE), respectively. Grade ≥3 treatment-related adverse events (TRAEs) occurred in 92% and 88% of patients, respectively.

For patients with 1 prior therapy (n=10) or ≥2 prior therapies (n=45), CR/CRi rates (95% CI) were 90% (55-100) and 67% (51-80); and median (95% CI) OS were not reached (NR; 2.1-NE) and 25.6 months (14.2-38.9), respectively. Incidence of Grade ≥3 TRAEs was 90% and 89%, respectively.

CR/CRi rates (95% CI) for patients with (n=25) and without (n=30) prior blinatumomab were 60% (39-79) and 80% (61-92). Median (95% CI) OS was 14.2 months (3.2-26.0) and NR (18.6-NE); and Grade ≥3 TRAEs occurred in 80% and 97% of patients, respectively.

For responders who did (n=10) or did not (n=29) receive subsequent alloSCT, median (95% CI) OS was NR (7.6-NE) and 38.9 months (18.6-NE).

Conclusion: Adults with R/R B-ALL benefitted from brexu-cel, regardless of age, number of prior therapies, prior blinatumomab exposure, or subsequent alloSCT. Survival was longer in patients with fewer prior therapies and in blinatumomab-naïve patients; however, small patient numbers and unmatched baseline characteristics limit interpretation of these results.

Three-Year Follow-up of Brexucabtagene Autoleucel (brexu-cel), an Anti-CD19 Chimeric Antigen Receptor (CAR) T-Cell Therapy, in Adults With Relapsed/Refractory B-cell Acute Lymphoblastic Leukemia (R/R B-ALL) in ZUMA-3

Shah B[1], Ghobadi A[2], Oluwole O[3], Logan A[4], Boissel N[5], Cassaday R[6], Leguay T[7], Bishop M[8], Topp M[9], Tzachanis D[10], O'Dwyer K[11], Arellano M[12], Lin Y[13], Baer M[14], Schiller G[15], Park J[16], Subklewe M[17], Abedi M[18], Minnema M[19], Wierda W[20], DeAngelo D[21], Stiff P[22], Jeyakumar D[23], **La Greca N[24]**, Zhang W[24], Adhikary S[24], Zhou L[24], Hadjivassileva T[24], Damico Khalid R[24], Houot R[25]
[1]Moffitt Cancer Center, Tampa, USA, [2]Washington University School of Medicine, St Louis, USA, [3]Vanderbilt-Ingram Cancer Center, Nashville, USA, [4]UCSF Medical Center, San Francisco, USA, [5]Hôpital Saint-Louis, Paris, France, [6]University of Washington School of Medicine and Fred Hutchinson Cancer Center, Seattle, USA, [7]Service d'hématologie clinique et thérapie cellulaire Hôpital du Haut-Leveque CHU de Bordeaux, Bordeaux, France, [8]The University of Chicago Medicine, Chicago, USA, [9]Universitätsklinikum Würzburg, Würzburg, Germany, [10]University of California, San Diego, San Diego, USA, [11]Wilmot Cancer Institute of University of Rochester, Rochester, USA, [12]Emory University School of Medicine, Atlanta, USA, [13]Mayo Clinic, Rochester, USA, [14]University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, USA, [15]David Geffen School of Medicine at UCLA, Los Angeles, USA, [16]Memorial Sloan Kettering Cancer Center, New York, USA, [17]Ludwig-Maximilians-Universität München, Munich, Germany, [18]University of California Davis Comprehensive Cancer Center, Sacramento, USA, [19]University Medical Center Utrecht, on behalf of HOVON/LLPC, Utrecht, The Netherlands, [20]The University of Texas MD Anderson Cancer Center, Houston, USA, [21]Dana-Farber Cancer Institute, Boston, USA, [22]Loyola University Chicago Stritch School of Medicine, Maywood, USA, [23]University of California Irvine Medical Center, Irvine, USA, [24]Kite, a Gilead Company, Santa Monica, USA, [25]Centre Hospitalier Universitaire de Rennes, Rennes, France

Aim: Brexu-cel is approved for R/R B-ALL in the US and Australia (≥18 years) and the EU (≥26 years). We report 3-year outcomes in ZUMA-3.

Method: Patients (≥18 years) had R/R B-ALL and received one infusion of brexu-cel (1×10^6 CAR T cells/kg). Primary endpoint was overall CR/CRi rate by central review.

Results: As of July 23, 2022, median follow-up in Phase 2 (N=55) was 38.8 months (range, 36.2-42.0). The overall CR/CRi, CR, and subsequent allogeneic stem cell transplant (alloSCT) rates were 71%, 56%, and 20%. Median (95% CI) DOR, censored and not censored at subsequent alloSCT, was 14.6 (9.4-24.1) and 18.6 months (9.6-23.6). Median (95% CI) relapse-free survival (RFS), censored and not censored at subsequent alloSCT, was 11.6 (2.7-20.5) and 11.7 months (2.8-20.5). At data cutoff, 22 patients were still alive (40%) with a median (95% CI) OS of 26.0 months (16.2-NE; N=55) and 38.9 months (26.0-NE) in patients with CR (n=31). OS rate at 36 months was 47.1% (95% CI, 32.7-60.2).

For Phase 1 and 2 patients treated at the pivotal dose (N=78), median follow-up was 41.6 months (range, 32.7-70.3). Median (95% CI) DOR censored and not censored at subsequent alloSCT was 18.6 (9.6-24.1) and 20.0 (10.3-24.1) months. Median (95% CI) RFS censored and not censored at subsequent alloSCT were both 11.7 (6.1-20.5) months. At data cutoff, 28 patients were still alive (36%) with median OS of 25.6 months (95% CI, 16.2-47.0; N=78).

The proportion of pooled Phase 1 and 2 patients with Grade ≥3 adverse events (AEs) that were deemed treatment-related was unchanged and no Grade 5 AEs occurred since the prior data cutoff.

Conclusion: With longer follow-up and expanded data set, responses remain durable in ZUMA-3, with a median OS of >3 years in Phase 2 patients with CR. Long-term safety analyses showed no new safety signals.

Efficacy of asciminib against ABL2 fusion-genes and the impact of the SH2/3 domains in asciminib treatment in high-risk Ph-like ALL.

Lagonik E[1,2], Page E[1,2,3], Forgione M[1,3], Eadie L[1,3], Yeung D[1,3,4,5], Hughes T[1,3,4,5], White D[1,2,3,4,6,7]
[1]Precision Medicine theme, SAHMRI, University of Adelaide, Adelaide, Australia, [2]Faculty of Sciences, Engineering and Technology, University of Adelaide, Adelaide , Australia, [3]Faculty of Health and Medical Science, University of Adelaide , Adelaide , Australia, [4] Australasian Leukaemia & Lymphoma Group, Richmond, Australia, [5]Royal Adelaide Hospital, Adelaide, Australia, [6] Australian & New Zealand Children's Haematology/Oncology Group, Clayton, Australia, [7] Australian Genomics Health Alliance, Parkville, Australia.

Aim: Ph-like Acute Lymphoblastic Leukaemia (ALL) is a high-risk subtype driven by a gene-expression profile similar to that of Ph^+ ALL, without the hallmark *BCR::ABL1* fusion-gene. Ph-like ALL includes *ABL1* and *ABL2* fusion-genes. The *ABL1/2* breakpoint often results in inclusion of the SH2/SH3 domains involved in Abl/Arg protein auto-inhibition. The *ABL2* fusion-genes maybe be sensitive to asciminib, a highly specific Abl allosteric inhibitor. Elucidating the efficacy of asciminib against *ABL2* fusion-genes and the impact of SH2/SH3 domains on asciminib sensitivity may expand the available treatments for high-risk Ph-like ALL patients.

Methods: The *PAG1::ABL2, RCSD1::ABL2* and *ZC3HAV1::ABL2* fusion genes were isolated from patient samples. Site directed mutagenesis was implemented on the *ZC3HAV1::ABL2* fusion for the construction of the Δ3 and Δ4 isoforms (absence of SH3 and SH2/SH3 domains, respectively). The myristate pocket was conserved in all constructs. The constructs were retrovirally transduced into cytokine-dependent Ba/F3 cells. Asciminib and ponatinib efficacy was assessed by Annexin V/7-AAD staining and the LD_{50} calculated as a measure of treatment-induced cytotoxicity. Statistical significance (P-value) was calculated by unpaired student's t-test.

Results: Ba/F3 cells expressing *ZC3HAV1::ABL2*, demonstrated sensitivity to asciminib with an LD_{50} of 175 nM ($p<0.0001$, compared to negative control). Deletion of SH3 and SH2/SH3 domains of *ZC3HAV1::ABL2* fusion-gene significantly increased the $LD_{50}^{asciminib}$ >10 μM, suggesting these domains are critical to asciminib-therapeutic effect. Similarly, lesions without the SH2/3 domains such as *PAG1::ABL2* and *RCSD1::ABL2*, had no sensitivity to asciminib (LD_{50} 25.4 and 27.2 μM respectively vs 24.8 μM negative control) (Figure 1 & 2). All cell lines exhibited sensitivity to ponatinib ($LD_{50}^{ponatinib}$ <2nM).
Conclusion: Asciminib was effective against the *ZC3HAV1::ABL2* fusion-gene *in vitro*, the only fusion-gene which harboured the SH2/SH3 domains. Moreover, the *ZC3HAV1::ABL2* Δ3 and Δ4 isoforms were not sensitive to asciminib. These data provide evidence that asciminib is effective against *ABL2* fusion-genes when the SH2/SH3 domains are present and an *in vivo* exploration is currently underway.

Figure 1: Asciminib LD_{50} cell death assay

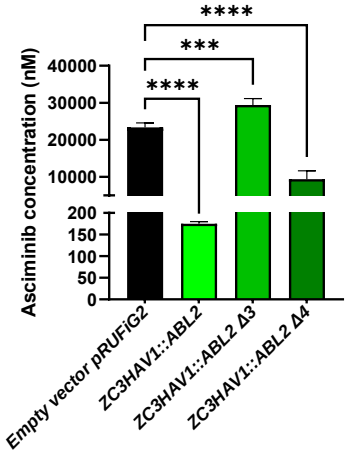
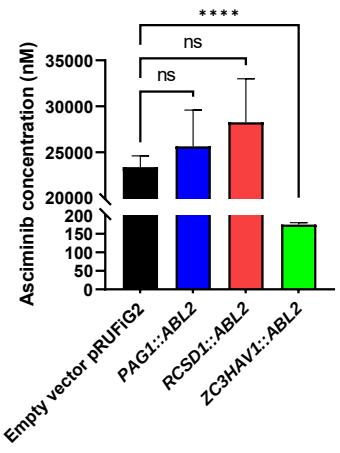


Figure2: Asciminib LD_{50} cell death assay



An audit of molecular measurable residual disease (MRD) testing in cases of acute myeloid leukaemia (AML) diagnosed in the Western Australian public health system (2019-2022).

Lewis K[1], Louw A, de Kraa R, de Saumarez S, Purtill D
[1]*Fiona Stanley Hospital, Perth, Australia*

Aim: Assessment of MRD is the standard of care in the management of AML. The European LeukemiaNet (ELN) MRD Consensus Document, first published in 2018 and updated in 2021, provides recommendations for standardised molecular MRD testing in AML. We performed a retrospective audit of AML cases diagnosed in the Western Australian public health system between 2019-2022 to determine the type and frequency of molecular MRD markers in this patient cohort and to assess clinician adherence to ELN MRD testing recommendations.

Method: Cases of acute myeloid leukaemia were identified from tertiary hospital bone marrow biopsy records and using Pathwest databases. Molecular MRD test results and patient demographics were obtained from the medical record and the Pathwest laboratory information system. Data collection included patient demographics, treatment type and site, ELN risk group, molecular MRD marker type and the frequency and timepoints of MRD testing. For patients undergoing intensive chemotherapy or allogenic stem cell transplant the timepoints for MRD testing were compared with the ELN guideline recommendations.

Results: 399 cases of newly diagnosed and relapsed AML were identified. Of these 199 (50%) had 1 or more molecular MRD markers detected during diagnostic investigations. Of these 199 patients, 109 (55%) of patients had MRD testing at one or more timepoints during their treatment. Concordance with molecular MRD testing was highest with locally tested markers (PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1). MRD markers with less well established timepoints and testing performed outside of Western Australia were requested less frequently.

Conclusion: Clinical tools to improve clinician adherence with requesting MRD testing at guideline recommended timepoints are required and are under development.

NGS-based clonality testing is a sensitive and complementary method for the diagnosis and monitoring of acute lymphoblastic leukaemia and other lymphoid malignancies

Ma S[1,4], Lin W[1,4], Hogan C[1], Fleming S[2], Fong C[3], Agarwal R[1]
[1]Austin Pathology, Heidelberg, Australia, [2]Alfred Hospital, Melbourne, Australia, [3]Austin Health, Heidelberg, Australia, [4]Co-first authors, ,

Aim: Next generation sequencing (NGS) based clonality testing for IgH and TCR gene rearrangements has been increasingly recognised to improve diagnostic accuracy in lymphoid malignancies. NGS based assays allow identification of the full range of clonal populations with underlying DNA sequences, thereby offering specific clonal markers and enhanced sensitivity for measurable residual disease (MRD) monitoring. Here, we interrogate the utility and accuracy of NGS in the workup of lymphoid malignancies and MRD monitoring of acute lymphoblastic leukaemia (ALL).

Method: All IGH and TCR rearrangement assays performed between 2016 and 2022 were retrospectively examined and correlated with patients' clinicopathologic details. Patients' DNA from blood, bone marrow and tissue samples were analysed on the LymphoTrack® Dx and MRD Assay platform (Invivoscribe, Inc) using Illumina® MiSeq.

Results: Diagnostic samples from 104 patients were tested. 41 of these samples were referred for clonality testing with the majority being in the context of suspected T cell lymphoproliferative disorders (LPD). Notably, T-LPD accounted for 58.3% of monoclonal cases (7/12), and polyclonal TCR rearrangement effectively excluded T-LPD. The remaining 63 diagnostic samples were performed for new ALL, where NGS detected clonality in 77.8% of cases (50/63). Of these, a total of 172 time points were assessed for MRD and demonstrated 83.1% concordance with flow cytometry. Nearly all discordance was due to NGS detecting disease below the threshold of detection for flow MRD. These results are clinically significant as they often herald disease relapse, thus help to guide management and pre-emptive intervention.

Conclusion: Our study concludes that NGS-based clonality testing is a powerful tool in assessing lymphocyte clonality. In ALL, it is also a more sensitive method in detecting MRD compared with flow cytometry in the majority of cases where IGH or TCR rearrangement is identified. Thus, NGS shows tremendous promise as an integral element in the diagnostic and management algorithms of lymphoid malignancies.

Investigating and therapeutically targeting the mutant cohesin complex in Down syndrome-associated acute megakaryoblastic leukaemia

Eyjólfsdóttir H[1], Thoms J[4], Subramanian S[4], de Bock C[6], Pimanda J[3,4,5], Ma D[7,1,2], Molloy T[1,2]
[1]St Vincent's Centre For Applied Medical Research, Sydney, Australia, [2]School of Clinical Medicine, UNSW Medicine & Health, St Vincent's Clinical Campus, Sydney, Australia, [3]School of Clinical Medicine, UNSW Medicine & Health, UNSW Sydney, Sydney, Australia, [4]School of Biomedical Sciences, Lowy Cancer Research Centre, UNSW Sydney, Sydney, Australia, [5]Department of Haematology, Prince of Wales Hospital, Sydney, Australia, [6]Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, Sydney, Australia, [7]Department of Haematology and Bone Marrow Transplant, St Vincent's Hospital Sydney, Sydney, Australia

Aim: Children with Down syndrome (DS) experience a 500-fold higher incidence of acute megakaryoblastic leukaemia (AMKL) than their non-DS counterparts. This is thought to be due to the interaction between the additional copy of chromosome 21 which characterises DS and one or more undefined driver mutations. We hypothesized that mutations in the cohesin complex, which regulates transcription factor (TF) binding site accessibility across the genome and is recurrently mutated in DS-AMKL, are key drivers of the malignancy. We aimed to investigate the pathways dysregulated by cohesin mutation as well as the potential for targeting them therapeutically.

Method: An *in vitro* model was developed in which a frameshift mutation in the cohesin member RAD21 was introduced by CRISPR into the wildtype AMKL cell like CMK. ChIP-Seq was used to investigate differences in binding events of several key haematopoietic TF located on chromosome 21. RNASeq was used to detect dysregulation of downstream regulatory pathways, and to guide selection of potential targeted therapies which were tested *in vitro*. Upregulation of drug targets, relevant pathways, and phenotypic changes were confirmed by QPCR, Western blotting, and flow cytometry.

Results: ChIP-Seq revealed several thousand new binding sites for multiple critical haematopoietic TFs became accessible as a result of RAD21 mutation. This in turn activated self-renewal and stem cell-like gene expression signatures in mutant cells and was accompanied by a significant loss of both stem cell markers CD34 and CD117, and markers of megakaryocyte maturation such as CD45, CD71, and polyploidy. Strong activation of the WNT and MAPK networks was observed, particularly in response to differentiation signals. Pharmacological inhibition of these pathways was highly effective in triggering cell death in RAD21-mutant AMKL cells.

Conclusion: This study has revealed critical new pathways that likely drive the pathogenesis of DS-AMKL and has suggested novel targeted therapeutic strategies for its treatment.

Myeloid malignancies with DDX41 variants in a real world Australian cohort

Nelles R[1], Aung H[1], Nandini A[1], Abdulrasool G[1], Lau C[1], Seymour L[1]
[1]Pathology Queensland, Brisbane, Australia

Aim: Myeloid neoplasms with germline DDX41 variants are an increasingly recognised cause of myeloid malignancy with an inherited predisposition. While there is a greater understanding of the pathogenesis and natural history of this condition uncertainty remains regarding the prognosis and outcomes of this group of patients.

The aim of this study was to review consecutive patients diagnosed with myeloid neoplasms with DDX41 mutations across Queensland in comparison to available literature.

Method: A retrospective review of all patients undergoing gene panel testing for myeloid malignancies performed across Pathology Queensland between September 2019 and May 2023 identified 21 unique patients with DDX41 mutations including 19 patients with germline and 2 with somatic only mutations. Data collected included baseline characteristics, curation of germline DDX41 variants, treatment/transplant decisions and follow up outcomes.

Results: The median age was 63 years old and affected predominantly male patients (81%). Presentations included AML (43%), MDS-IB2 (29%) or other MDS (29%). The most commonly identified germline DDX41 mutation was the M1I missense start loss substitution (24%), with the most common somatic DDX41 mutation being the R525H missense mutation (57%).

There were no significant differences in 2 year or median overall survival between AML, MDS-IB2 or other MDS with germline DDX41 mutations ($p>0.05$). A novel case of somatic DDX41 R525H mutation with high variant allele frequency (VAF 62%) due to copy neutral loss of heterozygosity of chromosome 5q (CN-LOH) was also identified in a patient with MDS with no other driver mutations.

Conclusion: This study identifies a unique Australian cohort with germline and somatic DDX41 mutations with a range of previously reported and novel germline mutations. This data adds to the growing body of evidence for the unique characteristics of patients with myeloid malignancy with inherited DDX41 variants.

Utilising Interphase Fluorescence in situ Hybridisation (FISH) in Rapid Diagnosis of Relapsed T-Lymphoblastic Leukaemia (T-ALL) with Phenotypic Evolution

Noor W[1], Hinds E[1], Diano G[1], Murphy N[1], Nelson N[1]
[1]*Royal Hobart Hospital, Hobart, Australia*

Background: Relapsed acute leukaemia may uncommonly result in clonal evolution and conversion of cell lineages. Distinguishing such phenotypic switch from the emergence of new therapy-related neoplasm has therapeutic and prognostic implications. Clarifying clonal relatedness by genomic analysis is often time consuming hence limiting its utility in disease entities that require urgent treatment.

Method: Interphase fluorescence *in situ* hybridisation (FISH) is a rapid turnaround test that was appropriately utilised to support the diagnosis of relapsed disease with phenotypic shift rather than a new therapy-related acute leukaemia which informed treatment decision-making. The clonal relationship was then confirmed with further genomic testing once available.

Case report: A 43-year old male was initially diagnosed in June 2022 with T-cell acute lymphoblastic leukaemia (T-ALL) and typical immunophenotype: icCD3$^+$, CD7$^+$, CD13$^+$, CD79a$^+$, TdT$^+$. FISH analysis revealed *TRA/D* gene rearrangement while molecular studies revealed *NOTCH1* p.(Phe1592_Arg1594delinsProLeuTrpGly), *EZH2* p.(Phe145Ser) and *PHF6* p.(Gly306Arg) variants. He was treated with 8 cycles of Hyper-CVAD A/B followed by POMP maintenance.

At 10 months following treatment, progressive thrombocytopenia prompted a repeat bone marrow evaluation revealed a large pleomorphic population of blasts (70%) with an absence of lineage-defining immunophenotype: CD34$^+$, CD117$^+$, CD33$^+$, CD13$^+$, HLA-DR$^+$, CD7$^+$, TdT$^-$, CD79a$^-$, MPO$^-$, icCD3$^-$, CD19$^-$.

Urgent FISH detected *TRA/D* (14q11.2) rearrangement supportive of disease relapse, which informed treatment decision making. Subsequent molecular and cytogenetic analysis confirmed the presence of the previously detected *NOTCH1*, *EZH2* and *PHF6* variants In addition, *TP53* and variant, der(16)t(1;16)(q21;q24) and add(9q34) confirming relapsed T-ALL with clonal evolution.

Conclusion: Relapsed acute leukaemia can be distinguished from a new therapy-related acute leukaemia by establishing clonal relationship. Rapid FISH testing was able to rapidly suggest clonal relatedness with original disease despite marked phenotypic alteration from original disease.

A ketogenic diet in acute myeloid leukaemia: feasibility, safety, and impact on chemotherapy efficacy.

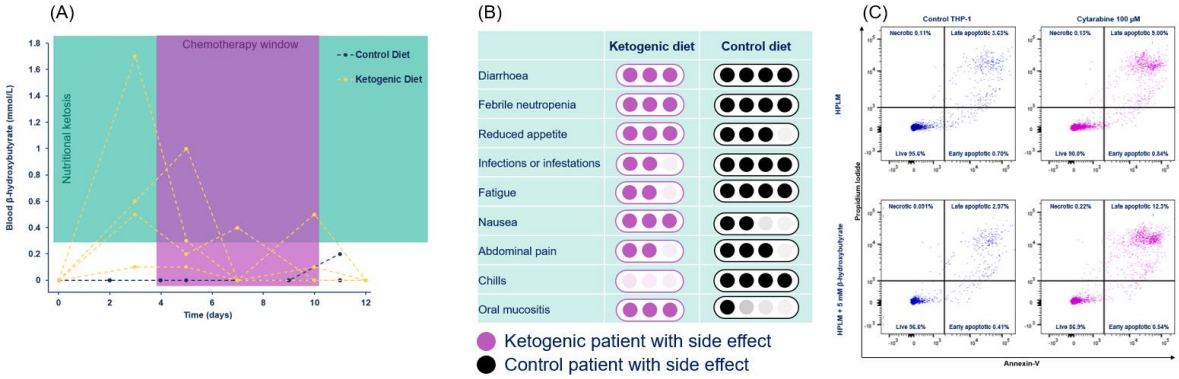
Pelaia T[1], Lastra Cagigas M[1,2,3], Rajakumar G[1,3], Skarratt K[1], Hayward R[1], Ardjmand A[1,2], Vaughan L[4,5,6], Fuller S[1,2], Fontana L[3,7,8]

[1]Sydney Medical School Nepean, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia, [2]Department of Hematological Oncology, Nepean Hospital, NBMLHD, Sydney, Australia, [3]Charles Perkins Centre, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia, [4]Crown Princess Mary Cancer Centre, Westmead Hospital, WSLHD, Sydney, Australia, [5]Institute of Clinical Pathology and Clinical Research, WSLHD, Sydney, Australia, [6]Lowy Cancer Research Centre, University of New South Wales, Sydney, Australia, [7]Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, Australia, [8]Department of Clinical and Experimental Sciences, Brescia University School of Medicine, Brescia, Italy

Aim: The LEU-KETO study aims to investigate the potential benefits of a ketogenic diet (KD) compared to standard care in reducing chemotherapy toxicity and other side-effects in acute myeloid leukaemia (AML) patients. We hypothesize that metabolic ketosis enhances chemotherapy efficacy in AML blasts and reduces toxicity in non-cancerous tissues by inhibiting the insulin/IGF-1/mTOR pathway. To test this, we evaluated the feasibility and safety of implementing a KD during induction chemotherapy and compared the incidence of side-effects between control and KD arms. Additionally, we used *in vitro* models to investigate chemotherapy-induced cellular damage in ketosis-mimicking environments.

Method: *In vivo:* Eligible *de novo* AML patients (*n*=8) were randomized to receive a control (*n*=4) or KD (*n*=4) upon admission to two Sydney hospitals. Biological samples, side-effects, and ketone body measurements for dietary compliance were collected daily during the ~1-month hospital stay. *In vitro:* The human monocytic AML cell line THP-1 was incubated with human-plasma like medium (HPLM) supplemented with a physiological concentration of ketone bodies (5 mM) for 24h prior to exposure to chemotherapy (cytarabine, 100 uM) for 24h. Apoptosis was measured by flow cytometry as a surrogate marker of chemotherapy efficacy.

Results:



Preliminary results show that (A) all patients (n=4) in the ketogenic group produced ketone bodies, unlike their control counterparts (n=4). (B) The incidence of side-effects was similar between the two groups, with a small decrease in infections in the ketogenic group, and no diet-induced complications. (C) *In vitro* analysis showed almost double the amount of apoptosis in cytarabine-treated THP-1 cells supplemented with ketones compared to control.

Conclusion: Preliminary results suggest that the KD is a feasible and safe nutritional intervention compatible with induction chemotherapy that could decrease post-chemotherapy side-effects in

AML patients. Ketone bodies sensitise AML cells to cytarabine, increasing apoptosis. Ongoing recruitment will provide further insight into the benefits.

Acute Myeloid Leukaemia (AML) in Queensland First Nations people

Kennedy G, Cossio D, Dhanda P, Birchley A, **Peters L**[1]

[1]*Cancer Alliance Qld, Woolloongabba, Australia*

Aim: To examine the incidence, treatment and outcomes of de novo AML among Queensland First Nations people.

Method: We obtained data on 1,319 people diagnosed with de novo AML between 2012-2021 from the Queensland Oncology Repository, in which 47 (3.6%) patients identified as First Nations people. We evaluated diagnostic testing, treatment patterns and survival outcomes.

Results: First Nations patients were younger (median age 54 vs 70 years) with males less overrepresented than the Non-First Nations cohort (51% vs 58% male). 94% of First Nations people underwent bone marrow aspirate and trephine (BMAT).

Rates of intravenous systemic therapy (IVST) were similar between First Nations people (74%) and Non-First Nations (68%).
Over the 10-year period, 11% of First Nations peoples and 26% of Non-First Nations people undergoing ST underwent bone marrow transplant (BMT) consolidation, however, this was not statistically significant (p=0.06).
30-day mortality following IVST was 17% for First Nations patients and 7% for Non-First patients (p=0.03). Overall survival across all treatment modalities was comparable (see Table 1).
Table 1: Kaplan-Meier survival estimates for treated patients

	1yr survival % (95%CI)	2yr survival % (95%CI)	5yr survival % (95%CI)
First Nations peoples	56 (38-71)	53 (35-68)	46 (29-62)
Non-First Nations peoples	63 (60-66)	49 (46-52)	33 (29-36)

Conclusion: This retrospective analysis of 10 years of Queensland data demonstrates similar rates of AML treatment approach and long-term outcomes in a non-age matched cohort of First Nations Australians in comparison to Non-First Nations patients. There was a trend toward lower rates of BMT among First Nations patients. The significance of this is unclear given the small patient cohort and other transplant related variables including induction mortality, donor options and patient preference.

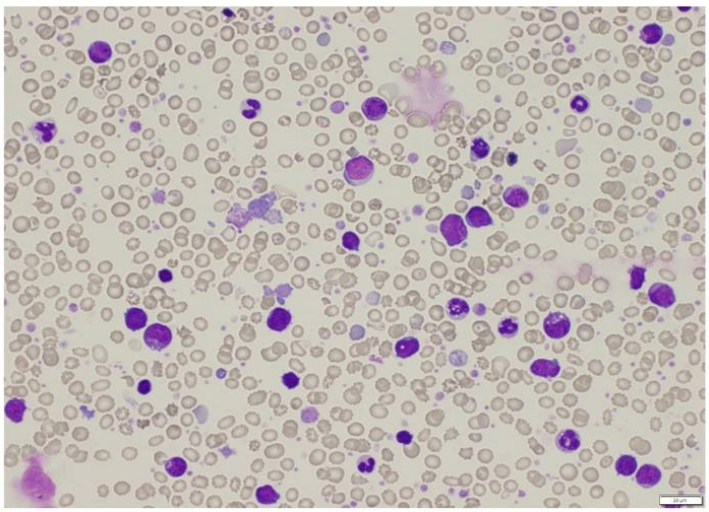
Transient abnormal myelopoiesis with non-constitutional trisomy 21 and GATA1 mutation

Reardon B[1,2], Ross B[1], Bateman C[1,3]
[1]The Children's Hospital at Westmead, Westmead, Australia, [2]Institute of Clinical Pathology and Medical Research, Westmead, Australia, [3]The Westmead Institute for Medical Research, Westmead, Australia

Case Report: We report a case of neonatal transient abnormal myelopoiesis (TAM) in a phenotypically normal premature neonate with non-constitutional trisomy 21 and GATA1 mutation. Emergency caesarean section was performed at 31 weeks gestation in a primigravid 28 year old woman with unremarkable antenatal care for abnormal cardiotocography. The neonate was hydropic with hepatomegaly, ascites and pericardial effusion with a widespread erythematous exanthem. Bloods revealed a haemoglobin of 48g/L, MCV 103fL, white cell count 54.0x10^9/L and platelets of 635x10^9/L. Blood film revealed leukoerythroblastosis with circulating blasts, platelet anisocytosis with megakaryocyte fragments (Image 1). A likely diagnosis of TAM was made. Peripheral blood cytogenetics revealed trisomy 21 on karyotype (47,XY,+21), with subsequent T-cell enriched lymphocyte culture showing a normal karyotype (46,XY), excluding Down syndrome (DS) mosaicism to a level of 5% (CI 95%). The child became increasingly clinically unstable with rapid progression and desquamation of rash (Image 2), and rise in white cell count to 107x10^9/L. Biopsy of the rash confirmed leukaemia cutis. Low dose cytarabine was commenced with rapid clinical and biochemical improvement. Later NGS testing revealed a GATA1 mutation (variant c.90_91del;p.(Val32Phefs*7). The neonate survived with later assessment demonstrating no phenotypic features of DS, but a persistent thrombocytopenia. Repeat bone marrow assessment and NGS testing have shown a normal karyotype and no detectable GATA1 mutation.

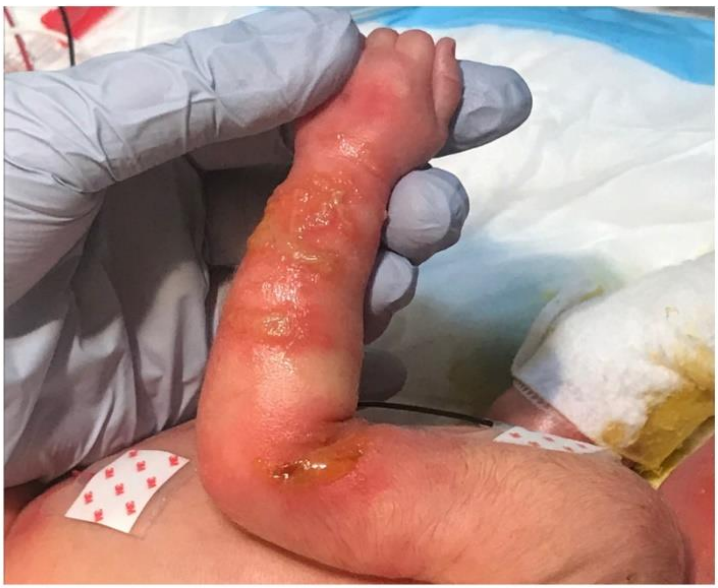
Discussion: TAM is a clonal proliferation of megakaryoblasts occurring in neonates, predominantly in those with DS, with mosaicism for trisomy 21 or with chromosome 21 translocations. Rarely, TAM may occur whereby the trisomy 21 may be found only within the blast population, with a single international case series finding only 17 cases over a 30 year period.[1] Somatic GATA1 mutations in TAM in neonates with DS have been reported in as much as 10% of cases with circulating blasts.[2] Our case represents a rare occurrence of non-constitutional trisomy 21 with concurrent GATA1 mutation who required treatment.

Image 1. Peripheral blood film.



Conclusion: TAM should be included in the differential for neonatal leukaemia even in a phenotypically normal infant.

Image 2. Cutaneous pustular lesions, leukaemia cutis.



References:

- Yuzawa K, Terui K, Toki T, et al. Clinical, cytogenetic, and molecular analyses of 17 neonates with transient abnormal myelopoiesis and nonconstitutional trisomy 21. Pediatr Blood Cancer. 2020 Apr;67(4):e28188
1. Maroz A, Stachorski L, Emmrich S, et al. GATA1s induces hyperproliferation of eosinophil precursors in Down syndrome transient leukemia. Leukemia. 2014;1259–1270

Impact of delay to intensive induction therapy on mortality in acute myeloid leukaemia in Queensland between 2012 and 2021.

Kennedy G, Cossio D, Dhanda P, Mollee P, **Rolfe T**
[1]*Cancer Alliance Qld, Woolloongabba, Australia*

Aim: This retrospective analysis of population data from a state-based cancer registry aims to evaluate impact of timing of commencing intensive induction chemotherapy on 30-day and 365-day mortality for de novo acute myeloid leukaemia (AML) patients in Queensland.

Method: Demographic, diagnosis, and treatment data for Queenslanders aged ≥15 years diagnosed with de novo AML between 2012 and 2021, treated in both public and private facilities were extracted from the Queensland Oncology Repository. The study included patients who underwent intensive induction chemotherapy for AML. Rates of 30- and 365-day mortality were reviewed.

Results: The review included 465 participants. Median time from diagnostic bone marrow biopsy to commencement of induction therapy was 4 days (IQR: 1-6 days). Increased age was associated with increased time between bone marrow biopsy and induction ($p<0.001$). Patients aged from 15 to 59 years commenced induction therapy within five days in 81% and 65% of those ≥60 years started treatment within the same timeframe.

Duration to induction therapy of greater than five days was not associated with statistically significant increase in 30-day or 365-day mortality. Mortality stratified by age is shown in Table 1.

Table 1: Mortality rates by time to treatment stratified by age

Days - BMAT to induction	**15-24yrs**			**25-59yrs**			**60-69yrs**			**70+yrs**		
	n	30-day mortality	365-day mortality	n	30-day mortality	365-day mortality	n	30-day mortality	365-day mortality	n	30-day mortality	365-day mortality
0-5	20	10%	20%	206	4%	20%	84	10%	37%	36	8%	44%
>5	4	0%	25%	50	4%	32%	45	0%	22%	20	0%	35%

Conclusion:
There is no evidence of an association between increased timeliness of induction and 365-day mortality for patients diagnosed with de novo AML based on this review of Queensland AML registry data.

CRLF2+ Acute Lymphoblastic Leukaemia: Beyond JAK/STAT Activation

Sadras T[1], Brown L[2], Salomon R[2], Jalud F[1], Li W[2], Ekert P[1,2]
[1]PETER MACCALLUM CANCER CENTRE, Melbourne, Australia, [2]Children's Cancer Institute, Sydney, Australia

B-cell acute lymphoblastic leukaemia (B-ALL) is a heterogeneous disease characterized by recurrent genomic aberrations that activate kinase signalling, disrupt tumour suppressors, and block B-cell differentiation. Lesions associated with B-ALL provide valuable prognostic insight, and can, in some cases allow administration of targeted agents (e.g. Tyrosine kinase inhibitors for BCR-ABL1+ B-ALL). Alterations that drive deregulated expression of cytokine receptor-like factor 2 (CRLF2) are present in 5–15% of paediatric and adult B-ALLs and represent a group of patients with particularly poor prognosis and high-rates of relapse. These patients respond poorly to standard chemotherapy, and currently lack effective targeted therapies.
Approximately 50% of CRLF2+ B-ALLs harbor activating mutations in JAK2, most commonly JAK2-R683S/G. Co-expression of CRLF2 and mutant JAK2 results in constitutive STAT5 activation, and factor-independent transformation of B-cell progenitors. The current consensus is that activated JAK/STAT activation is the hallmark of CRLF2 B-ALL, however JAK2 inhibitors such as Ruxolitinib have shown very limited efficacy in this leukemia. Our work and that from others, shows that some CRLF2+ B-ALLs lacking JAK2 mutations instead harbor activating mutations in the RAS-ERK pathway (e.g. KRAS-G12D). Using single-cell approaches we show here that in rare cases of patients with both STAT and ERK activating lesions, these mutations are present in competing clones. This highlights that therapeutic approaches for CRLF2+ B-ALL are inherently more complex, and it remains unknown how sub-clonal mutations alter the signalling properties and drug responses of CRLF2+ leukemias. To test this, we have established murine models expressing the human CRLF2 receptor complex and common JAK2 and RAS pathway mutations. We show for the first time that the combination of CRLF2 with RAS mutations behaves distinctly to the combination of CRLF2 and JAK2, and drives unique drug dependencies which can be therapeutically leveraged.

High expression of ENO1 and low levels of circulating anti-ENO1 autoantibodies correlate with MDS/AML

Scorgie F[1,2], Theron D[2,3], Barry D[2,3], Cox K[1], Sillar J[1,4], Enjeti A[1,2,3,4], Skelding K[2,3], Lincz L[1,2,3]
[1]Calvary Mater Newcastle, Waratah, Australia, [2]Hunter Medical Research Institute, New Lambton Heights, Australia, [3]University of Newcastle, Callaghan, Australia, [4]NSW Health Pathology-Hunter, New Lambton Heights, Australia

Aim: In solid tumours, high expression of the glycolytic enzyme, alpha-enolase (ENO1), predicts for poor patient overall survival (OS), and circulating autoantibodies to ENO1 correlate positively with diagnosis and negatively with advanced disease. Although *ENO1* is one of the most highly expressed genes in AML, its potential role as a biomarker has not been investigated. The aim of this study was to determine if ENO1 mRNA, protein, and/or autoantibodies are altered in AML/MDS and correlate with patient outcomes.

Method: A meta-analysis was performed using AML patient OS curves comparing high and low *ENO1* gene expression available through online databases (cBioPortal, PrognoScan, PRECOG). Immunohistochemistry for ENO1 was performed on bone marrow trephines from 22 AML/MDS patients and quantified using QuPath. Plasma anti-ENO1 antibodies were measured by ELISA in 64 AML/MDS patients and 18 controls. Cohorts were compared by Kruskal-Wallis tests with Dunn's post hoc analysis using MedCalc statistical software. P-values <0.05 were considered statistically significant.

Results: A meta-analysis of 9 AML datasets (n=1434 patients) indicated that high *ENO1* gene expression predicts for poor OS (HR=1.3, 95%CI: 1.2-1.5, p<0.001). Additionally, when compared to AML in remission (n=5), ENO1 protein was significantly over-expressed at diagnosis in bone marrow from both AML (n=5, p<0.01) and MDS patients (n=12, p<0.05), and did not correlate with percentage of blasts (rho=0.28, p=0.21). AML patients had significantly lower circulating levels of ENO1 autoantibodies compared to controls (median, range=1.5, 0.0 - 36.9 vs 11.2, 0.0 - 40.2 ng/ml, p=0.002), with a positive test for trend (controls>MDS diagnosis>MDS transfusion dependent>AML, p=0.0004). There was no significant difference in OS between AML patients with high vs low levels of ENO1 autoantibodies (n=34, p=0.71).

Conclusion: BM immunostaining for ENO1 could aid in diagnosis of MDS, while individual patient monitoring of anti-ENO1 autoantibody levels may be a biomarker for disease progression.

An unusual case of acute promyelocytic leukaemia

Sidhu A[1], Lovelock D[1], Keng T[1], Klose N[2], Harvey Y[1], Joy C[1], Boxall G[3], Supple S[4], Phan H[1]
[1]Sullivan Nicolaides Pathology, Bowen Hills, Australia, [2]Brisbane Blood Specialists, Greenslopes, Australia, [3]Pathology Queensland, Woolloongabba, Australia, [4]NSW Pathology, Camperdown, Australia

Aim: Acute promyelocytic leukaemia (APL) is a subtype of acute myeloid leukaemia (AML) which accounts for around 10% of all AML diagnoses. Definitive diagnosis of APL requires detection of the *PML::RARA* translocation by fluorescence *in situ* hybridization (FISH) and/or reverse transcriptase polymerase chain reaction (RT-PCR). Rarely, in some patients, this can be challenging due to unusual rearrangements making the translocation cryptic to one or both of these methodologies and further testing is required to confirm the diagnosis. We will address some of the challenges associated with the diagnosis of APL with unusual gene rearrangements, such as demonstrated in this instance.

Method: Retrospective case study of a patient presenting with pancytopenia and occasional abnormal promyelocytes.

Results: A 49-year-old male presented to his GP with dyspnoea, epistaxis, bruising and a petechial rash. Full blood count (FBC) showed pancytopenia, with mild left shift and occasional abnormal bilobed promyelocytes. Urgent FISH for t(15::17) *PML::RARA* and flow cytometry was requested and the patient was referred urgently to hospital. Initial FISH studies for *PML::RARA* and *RARA* breakapart were negative. A bone marrow aspiration and trephine was subsequently performed which confirmed the diagnosis of AML based on 65% blasts. These blasts were not typical for APL, demonstrating heavy basophilic granulation, with no bundles of Auer rods or abnormal bilobed promyelocytes observed. The blasts were positive for CD13, CD33, MPO and negative for CD34, HLA-Dr and TdT. Subsequent RT-PCR testing confirmed the presence of the *PML::RARA* rearrangement.

Conclusion: The diagnosis of APL represents a medical emergency and timely laboratory diagnosis is essential. However, our case was unusual due to atypical morphologic findings and negative initial FISH results for *PML::RARA*. It is important to be aware that both a delayed diagnosis, or a missed diagnosis, can have catastrophic outcomes for these patients.
No conflicts of interest to disclose.

Optimising treatment of older persons with Acute Myeloid Leukaemia through the implementation of diagnostic and longitudinal frailty assessments.

Simonsen C[1], Ling Y[1,2,3,4], Hubbard R[1,4], Berry R[1], Costin E[1], Sia A[1,4], Shafiee-Hanjani L[4], Shaw A[4], Tate C[1,3,4], Mollee P[1,4], Marlton P[1,4], Bird R[1], McPherson I[1]
[1]Princess Alexandra Hospital, Brisbane, Australia, [2]QIMR, Brisbane, Australia, [3]Pathology Queensland, Brisbane, Australia, [4]University of Queensland, Brisbane, Australia

Aim: Acute Myeloid Leukaemia (AML) is an aggressive blood cancer predominately affecting older people. Frailty is consistently associated with poor outcomes, yet not uniformly measured objectively in treatment decision making, potentially exposing some patients to toxic therapies without meaningful benefit. We aimed to determine the utility of objective frailty assessments utilizing the Frailty Index (FI) in older patients with AML.

Methods: This retrospective audit included patients diagnosed with AML >60 years of age from September 2022 – May 2023 with FI assessments performed at baseline. In previous studies a FI score >0.25 was determined to indicate significant frailty (McCarthy, et al. 2018, BMC Cancer

Results: Sixteen patients were included (median age 74 years, range 63-90). Most patients were frail at baseline (median FI 0.37, range 0.09–0.71). Five patients received intensive chemotherapy (IC), 7 received lower intensity therapies (venetoclax-based) and 4 received best supportive care (BSC). All patients who received lower intensity therapies (7/7) were frail, whereas 2/5 who received IC were frail. With limited follow-up, there were no significant survival differences between groups according to frailty (FI above and below 0.25) and treatment intensity. Baseline ECOG ranged from 0-3, median 1.5. Overall, there was correlation between ECOG and FI, especially at ECOG 2 and greater, however a significant range of FIs (0.09-0.39) was measured for patients with ECOG 1 (n=7). A subset of 10 patients had an additional FI subsequent to diagnosis. Only 1 patient experienced a reduction in frailty with therapy (74 year old male treated with venetoclax / azacitidine). No patients became non-frail.

Conclusion: The prevalence of frailty is significant in AML populations >60 years of age, which continues despite AML-directed therapy. ECOG is non-discriminatory for frailty, especially at ECOG 1. The preliminary findings of this ongoing study demonstrate the need for personalised treatment decisions in frail patients, particularly in those with ECOG scores 0-1 at baseline.

KMT2A amplification in B lymphoblastic leukaemia

Tegg E[1], Wren C[1]

[1]*Nsw HP, Westmead, Australia*

Aim: *KMT2A* gene rearrangements are known to occur in both myeloid and lymphoid leukaemia. *KMT2A* amplification is rarer and generally reported to occur in only 1% of cases of acute myeloid leukaemia (AML) and is rarely reported in B-lymphoblastic leukaemia (B-ALL) with only a handful of cases in the literature.

Method: We present a 69 year old male who presented to the emergency department with symptoms of melena, coffee ground vomit, syncope and confusion, on a background of 2 weeks of abdominal pain. A full blood count showed he was pancytopenic with 17% circulating blasts. Flow cytometry and rapid fluorescence in situ hybridization (FISH) testing for *BCR::ABL1* gene fusion and a *KMT2A* rearrangement were performed on the peripheral blood to establish the blast cell lineage and further classify the leukaemia. Further FISH testing was also performed for TP53 gene deletion.

Results: Flow cytometry testing showed that the blasts were positive for CD45(dim),CD19, CD34, CD38, CD15(variable) and HLA-DR. They were negative for CD3, CD4,CD5, CD7, CD10, CD13,CD20,CD33,CD56,CD64,CD117. Additional markers showed the blasts to be positive for intracellular CD79a, CD22 and to be negative for MPO and intracellular CD3, confirming that the diagnosis was B-ALL. The FISH showed *KMT2A* amplification with >20 signals per cell. There was no evidence of *BCR::ABL1* gene fusion; however, there were 3~4 copies of *BCR* and 3 copies of *ABL1* suggesting aneuploidy for chromosomes 9 and 22.

Conclusion: This is a fascinating case of *KMT2A* amplification with associated *TP53* gene deletion and clonal evolution in adult de novo precursor B-ALL.

Mutational profiling of NPM1-mutated AML

Thomas J[1], Aung H[1], Nandini A[1], Nelles R[1], Ling Y[1], Seymour L[1]
[1]*Pathology Queensland, Brisbane, Australia*

The presence of *NPM1* mutation confers the diagnosis of AML with mutated *NPM1* and is known to be associated with a favourable prognosis. The recently updated WHO and ICC classification guidelines (2022) identified a group of genes as myelodysplasia-related (MR) genes, when mutated are associated with adverse risk in AML (ELN 2022). These include *ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2* and *RUNX1* (ICC only). Previous studies have shown that most of the *NPM1* mutated AML have concurrent gene mutations. Whether the presence of MR gene mutations can alter the otherwise favourable prognosis of *NPM1*-mutated AML has become the focus for some of the recently published studies. Here, we retrospectively assess all AML cases tested at Pathology Queensland with NGS data available. This cohort includes a total of 81 *NPM1*-mutated AML with mutational profiling done by NGS-based assay across 37 genes that are of clinical relevance in myeloid malignancies. In line with the previous studies, our results also showed that all *NPM1*-mutated AML have at least one additional mutation/variant (ranging between 1 to 6 per case) and most were detected at >5% variant allele frequency (VAF). Out of 81 AML with mutated *NPM1*, only 15 cases have MR gene mutations, representing ~18% of all *NPM1*-mutated AML. This is compared to the presence of MR gene mutations in up to 47% of non-*NPM1* mutated AML in the same cohort. All 15 cases have only a single MR gene mutation with *SRSF2* being the most mutated gene (seen in 8 cases), followed by *STAG2* (5 cases), *EZH2* (1 case) and *ZRSR2* (1 case). Overall, this study confirms reduced, but significant co-occurrence of MR genes in *NPM1*-mutated AML. Further studies will determine the prognostic influence in this favourable subtype.

Judicious use of precise FISH panels guided by population prevalence may assist pragmatic diagnosis of Ph-like ALL - A Systematic Review

Thompson J[1,4], Thompson G[3,4], White D[1,4], Yeung D[1,2,4]
[1]South Australian Medical Research Institute, Adelaide, Australia, [2]Royal Adelaide Hospital, Adelaide, Australia, [3]Women's and Children's Hospital, Adelaide, Australia, [4]The University of Adelaide, Adelaide, Australia

Aim: Diagnosis of Philadelphia chromosome like ALL (Ph-like ALL) in the real-world remains challenging because of definitional complexities, the diverse diagnostic techniques available and the cost, skill and time involved. We summarise evidence for diagnosis of Ph-like ALL using fluorescent in-situ hybridization (FISH) targeting only clinically important and actionable lesions, an accessible and cost-effective diagnostic technique.

Method: Electronic databases were interrogated using broad MESH terms for articles reporting a detailed FISH strategy for diagnosis of Ph-like ALL published since 2014, yielding 653 full text articles and abstracts. We searched National Library of Medicine Databases including PubMed, Medline, Embase, Cochrane and relevant abstracts. We included studies with a primary aim of determining the utility of FISH for Ph-like ALL diagnosis and studies with broader aims demonstrating Ph-like ALL diagnostic algorithms which partially involved FISH.

Results: Nineteen studies met inclusion criteria. Evidence for FISH to detect *CRLF2* rearrangements in Ph-like ALL is strongly established and evidence for FISH to detect non-*CRLF2* lesions is evolving rapidly. We documented 1620 non-*CRLF2* Ph-like diagnostic FISH published results. Confirmatory side-by-side methods were applied in 6 studies (246 samples), 4 of which demonstrated 100% concordance of FISH results with alternative methods, while two studies demonstrated over 70% sensitivity and specificity. Additional studies demonstrated wide utilisation of FISH in Ph-like ALL classification across diverse geographies and ethnicities, with contrasting prevalence, implicating a need for targeted FISH strategies.

Conclusion: In real-world cohorts, it may be clinically useful to prioritise limited early FISH in B-ALL diagnostic algorithms to identify Ph-like abnormalities that respond to locally available kinase inhibitors to promote and prioritise broad access to effective targeted treatment. Additional studies are required to provide adequately powered validations and verifications of targeted Ph-like FISH panels to confirm sensitivity and specificity against side-by-side gold standard methods, and to define optimal local approaches.

Novel use of targeted BCL-2 inhibition as part of combination therapy is a promising strategy in aggressive relapsed-refractory haematological malignancies – A case series.

Tong M[1], Aung W[1], Baskaran A[1], Nguyen D[1], Hua M[1]
[1]*Liverpool Hospital, Sydney, Australia*

The selective BCL-2 inhibitor venetoclax has proven efficacy as part of combination therapy in acute myeloid leukaemia[1, 2] and chronic lymphocytic leukaemia.[3] There is limited data in its usage in other haematological malignancies. We present three cases where the use of compassionate access venetoclax in combination with chemotherapy resulted in remission in aggressive relapsed-refractory haematological malignancies, highlighting the promising potential of this medication. Case 1 is a 47-year-old female with relapsed stage IV blastoid mantle cell lymphoma post BEAM autograft (carmustine, etoposide, cytarabine, melphalan) after induction with the Nordic protocol. She achieved remission with venetoclax alongside DHAP (dexamethasone, cisplastin, cytarabine) and proceeded to allogeneic stem cell transplant. Case 2 is a 37-year-old female with multiply relapsed Philadephia-negative B acute lymphoblastic leukaemia, having progressed through hyperCVAD (dexamethasone, cyclophosphamide, doxorubicin, vincristine, methotrexate, cytarabine) with POMP maintenance (prednisolone, mercaptopurine, methotrexate, vincristine), blinatumomab, inotuzumab and FLAG reinduction (fludarabine, cytarabine). She achieved remission with venetoclax alongside ALL06 and rituximab and proceeded to allogeneic stem cell transplant. Case 3 is a 42-year-old male with multiply relapsed plasmablastic myeloma, having progressed through VRD induction (bortezomib lenalidomide dexamethasone), DPACE salvage (dexamethasone, cisplatin, etoposide, cyclophosphamide, doxorubicin), DVD (daratumumab, bortezomib, dexamethasone) and a BEAM autograft. He achieved short-lived remission with venetoclax alongside hyperCVAD.

These cases highlight the promising utility of targeting BCL-2 in aggressive haematological malignancies that have otherwise failed standard of care treatments. Given the importance of anti-apoptotic pathway mutations in the survival of malignant cells and their development of chemotherapy resistance,[4-7] there is an ongoing need for further research to develop additional therapeutic strategies to effectively target these pathways and venetoclax represents an exciting treatment option.

References

1. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. N Engl J Med. 2020;383(7):617-29.
2. Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Laribi K, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. Blood. 2020;135(24):2137-45.
3. Fischer K, Al-Sawaf O, Bahlo J, Fink A-M, Tandon M, Dixon M, et al. Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. N Engl J Med. 2019;380(23):2225-36.
4. Perini GF, Ribeiro GN, Pinto Neto JV, Campos LT, Hamerschlak N. BCL-2 as therapeutic target for hematological malignancies. J Hematol Oncol. 2018;11(1):65.
5. Valentin R, Grabow S, Davids MS. The rise of apoptosis: targeting apoptosis in hematologic malignancies. Blood. 2018;132(12):1248-64.
6. Wu H, Medeiros LJ, Young KH. Apoptosis signaling and BCL-2 pathways provide opportunities for novel targeted therapeutic strategies in hematologic malignances. Blood Rev. 2018;32(1):8-28.
7. Yalniz FF, Wierda WG. Targeting BCL2 in Chronic Lymphocytic Leukemia and Other Hematologic Malignancies. Drugs. 2019;79(12):1287-304.

Establishment of a NPM1 Mutation Copy Number Estimator for Xpert® NPM1 Mutation Test

Zhao Y[1], Wei H[1]
[1]Cepheid, Sunnyvale, United States

Aim: The nucleophosmin (*NPM1*) is the most mutated gene (~30%) in Acute Myeloid Leukemia (AML)[1]. Three NPM1 mutations (type A, B, and D) represent ~84% in NPM1-mutated AML cases while other uncommon subtypes occupy ~16%[2]. Xpert® NPM1 mutation, an automated cartridge-based test for measuring NPM1 mutation transcript levels (type A, B and D), is standardized to quantify the amount of mutated NPM1 relative to ABL1 control gene based on delta Ct in peripheral blood[3]. Since mutated NPM1 level is crucial for risk assessment, medication selection, and ongoing therapeutic monitoring in AML[4,5], it is important to obtain the NPM1 mutation copy number (CN). The aim of this work is to develop NPM1 mutation CN estimator and to compare %NPM1 mutation/ABL1 reporting between delta Ct-based and CN-based methods.

Method: Five levels of NPM1 mutations (A, B, D) and ABL1 IVT-RNA panels as well as two lots of Xpert® NPM1 mutation tests were used to generate standard curves for CN and %CN reporting. The cell lysates from cell lines carrying either NPM1 mutation A, B, or D and AML clinical samples containing NPM1 mutations were examined to evaluate the CN and %CN between two lots of the Xpert® NPM1 mutation tests and to compare the delta Ct-based and CN-based methods for reporting %NPM1 mutation/ABL.

Results: Linearity was demonstrated in Ct vs CN input for NPM1 mutation and ABL1 with R^2 above 0.96 for Lot1 and Lo2. Less than 3-fold difference was exhibited for CN and %CN across two lots of Xpert® NPM1 mutation test. Less than 3-fold difference was observed in %NPM1 mutation/ABL1 reporting between delta Ct-based and CN-based approaches.

Conclusion: An NPM1 mutation copy number estimator for Xpert® NPM1 mutation test was established, which will provide diagnostic and prognostic values for NPM1-mutated AML patients.

Image:

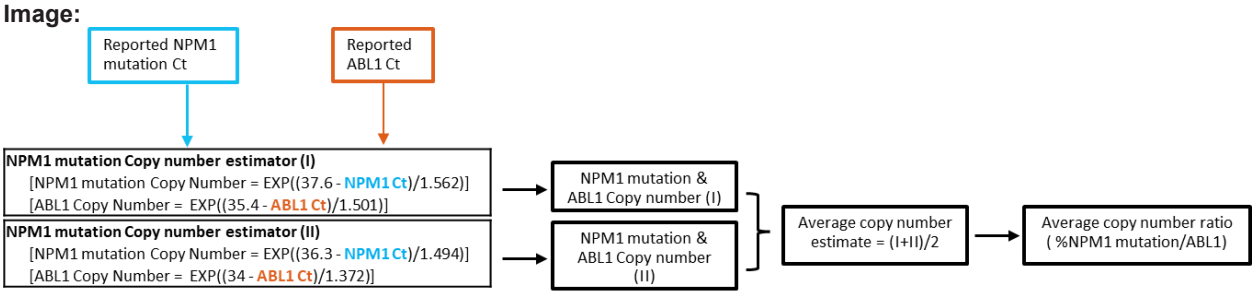


Figure 1: Two sets of NPM1 mutation copy number estimator for Xpert® NPM1 mutation test, which will provide diagnostic and prognostic values for NPM1-mutated AML patients. Enter reported NPM1 mutation Ct and ABL1 Ct into the formulas (I) and (II) to calculate the copy number. Average copy number of NPM1mutation and ABL1 from both formulas will be utilized in obtaining averaged %NPM1 mutation/ABL1.

References:

- Brunangelo Falini, Lorenzo Brunetti, Paolo Sportoletti, Maria Paola Martelli; NPM1-mutated acute myeloid leukemia: from bench to bedside. *Blood* 2020; 136 (15): 1707–1721.

1. Patkar N, Kodgule R, Kakirde C, Raval G, Bhanshe P, Joshi S, Chaudhary S, Badrinath Y, Ghoghale S, Kadechkar S, Khizer SH, Kannan S, Shetty D, Gokarn A, Punatkar S, Jain H, Bagal B, Menon H, Sengar M, Khattry N, Tembhare P, Subramanian P, Gujral S. Clinical impact of measurable residual disease monitoring by ultradeep next generation sequencing in NPM1 mutated acute myeloid leukemia. Oncotarget. 2018 Nov 27;9(93):36613-36624. doi: 10.18632/oncotarget.26400. Erratum in: Oncotarget. 2019 Jan 15;10(5):617. PMID: 30564301; PMCID: PMC6290958.
2. Xpert® NPM1 mutation brochure.
3. Forghieri, Fabio et al. “Minimal/Measurable Residual Disease Monitoring in NPM1-Mutated Acute Myeloid Leukemia: A Clinical Viewpoint and Perspectives.” International journal of molecular sciences vol. 19,11 3492. 6 Nov. 2018
4. Network NCC. Acute Myeloid Leukemia, Version 2.2023, NCCN Clinical Practice Guidelines in Oncology. Acute Myeloid Leukemia. Online: NCCN; 2023

Establishment of a PML-RARa Copy Number Estimator for Prototype Xpert® PML-RARa Assay

Liu M[1], Zhao Y[1], Liu Y[1], **Wei H[1]**, Saini J[1], Goldman D[1], Kayhan S[1], Tran T[1], Yuan L[1], Day G[1]
[1]*Cepheid, Sunnyvale, United States*

Aim: Acute Promyelocytic Leukemia (APL) represents 10-15% of Acute Myeloid Leukemia (AML). The PML-RARa fusion transcript is expressed in more than 95% of APL patients. Three PML-RARa isoforms (bcr1, bcr2, and bcr3) are identified in 90-95% of PML-RARa positive cases. Prototype Xpert® PML-RARa, an automated cartridge-based assay for measuring PML-RARa fusion transcript levels (bcr1, bcr2, and bcr3), is standardized to quantify the amount of PML-RARA relative to ABL1 control gene based on delta Ct in peripheral blood. Since PML-RARa level is crucial for diagnosis and ongoing therapeutic monitoring in APL, it can be useful to obtain the PML-RARa copy number (CN). The aim of this work is to develop PML-RARa CN estimator and to compare %PML-RARa/ABL1 reporting between delta Ct-based and CN-based methods.

Method: Four levels of PML-RARa (bcr1, bcr2, and bcr3) and ABL1 IVT-RNA panels as well as two lots of prototype Xpert® PML-RARa assay were used to generate standard curves for CN and %CN reporting. The samples with spiked-in bcr1 IVT-RNA and APL clinical samples containing PML-RARa fusion transcript were examined to evaluate the CN and %CN between two lots of the prototype Xpert® PML-RARa assay and to compare the delta Ct-based and CN-based methods for reporting %PML-RARa/ABL1.

Results: Linearity was demonstrated in Ct vs CN input for PML-RARa (R^2>0.98) and ABL1 (R^2>0.97). Less than 2-fold difference was exhibited for CN and %CN across two different lots. Less than 2-fold difference was observed in %PML-RARa/ABL1 reporting between delta Ct-based and CN-based approaches.

Conclusion: A PML-RARa copy number estimator for prototype Xpert® PML-RARa assay was established, which will provide helpful information for diagnosis and prognosis of APL. (Product in development. Not for use in diagnostic procedures. Not reviewed by any regulatory body.)

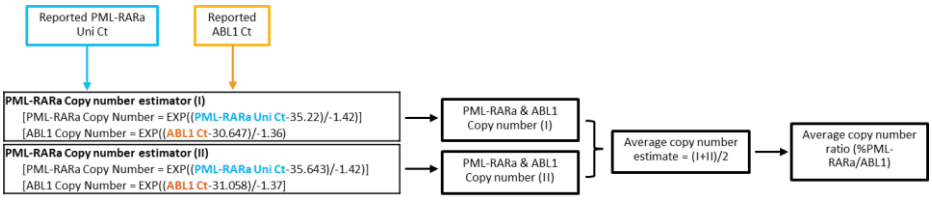


Figure 1: Two sets of PML-RARa copy number estimator for prototype Xpert® PML-RARa assay, which will provide diagnostic and prognostic values for APL. Enter reported PML-RARa Uni Ct and ABL1 Ct into the formulas (I) and (II) to calculate the copy number. Average copy number of PML-RARa and ABL1 from both formulas will be utilized in obtaining averaged %PML-RARa/ABL1.

Establishment of a BCR-ABL p190 Copy Number Estimator for Xpert® BCR-ABL Ultra p190 Test

Liu M[1], Zhao Y[1], Liu Y[1], **Wei H[1]**, Tran T[1], Yuan L[1], Day G[1]
[1]*Cepheid, Sunnyvale, United States*

Aim: BCR-ABL p190 (e1a2) is expressed in 20-30% of Acute Lymphoblastic Leukemia (ALL) and 1-2% of Chronic Myeloid Leukemia (CML). The co-expression of p190 and p210 is rare in both CML and ALL. Xpert® BCR-ABL Ultra p190, an automated cartridge-based test for measuring BCR-ABL p190 transcript level, is standardized to quantify the amount of BCR-ABL p190 relative to ABL1 control gene based on delta Ct in peripheral blood of patients. Since BCR-ABL p190 level is crucial for risk stratification and treatment decisions in ALL as well as diagnosis and continuous therapeutic monitoring in CML, it can be useful to obtain the copy number (CN) of BCR-ABL p190. The purpose of this study is to establish a BCR-ABL p190 CN estimator with known CN of e1a2-ABL IVT-RNA as well as to compare %BCR-ABL p190/ABL1 reporting between delta Ct-based and CN-based methods.

Method: Nine levels of e1a2-ABL IVT-RNA as well as two lots of Xpert® BCR-ABL Ultra p190 tests were used to generate standard curves for CN and %CN reporting. BCR-ABL p190 contrived samples and ALL clinical samples containing the BCR-ABL p190 transcript were examined to evaluate the CN and %CN between two lots of the Xpert® BCR-ABL Ultra p190 tests and to compare the delta Ct-based and CN-based methods for reporting %BCR-ABL p190/ABL1.

Results: Linearity was demonstrated in Ct vs CN input for BCR-ABL p190 (R^2>0.99) and ABL1 (R^2>0.98). Less than 1.4-fold difference was exhibited for CN and %CN across two different lots. Less than 2-fold difference was observed in %BCR-ABL p190/ABL1 reporting between delta Ct-based and CN-based approaches.

Conclusion: A BCR-ABL p190 copy number estimator for Xpert® BCR-ABL Ultra p190 test was established, which will provide helpful information for diagnosis and prognosis of diseases relating to BCR-ABL p190.

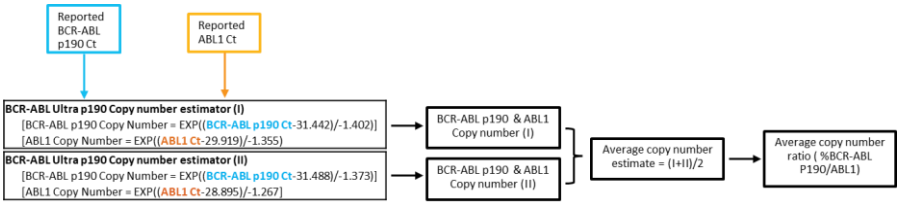


Figure 1: Two sets of BCR-ABL Ultra p190 mutation copy number estimator for Xpert® BCR-ABL Ultra p190 tests, which will provide helpful information for diagnosis and prognosis of diseases relating to BCR-ABL p190. Enter reported BCR-ABL p190 Ct and ABL1 Ct into the formulas (I) and (II) to calculate the copy number. Average copy number of BCR-ABL p190 and ABL1 from both formulas will be utilized in obtaining averaged %BCR-ABL p190/ABL1.

Improved MRD negativity rates in adverse genomic risk B-ALL patients with chemotherapy / blinatumomab induction: experience from the Australasian Leukaemia Lymphoma Group (ALLG) ALL06/09 studies.

White D[1,2,3], Rehn J[1,3], Schutz C[1], Heatley S[1,3], Eadie L[1,3], Thomson A[1,3], Page E[1,3], Osborn M[1,2,3,4], Gangatharan S[2,5], Ng A[2,6], Fleming S[2,7,8], Fedele P[2,8], Trahair T[2,9], Casey J[2,10], Mapp S[2,11], Cheung C[2,12], Armytage T[2,13], Henderson M[2,14], Sutton R[2,14], McCloud P[2,15], Larsen S[2,16], Presgrave P[2,17], Kwan J[2,18], Bennett S[2,19], Fong C[2,20], Dalla-Pozza L[2,21], Greenwood M[2,22,24], Yeung D[1,2,3,23,24]

[1]SAHMRI - Precision Cancer Medicine Theme, Adelaide, Australia, [2]Australasian Leukaemia Lymphoma Group (ALLG), Melbourne, Australia, [3]Faculty of Health and Medical Sciences. The University of Adelaide, Adelaide, Australia, [4]Women's and Children's Hospital, Adelaide, Australia, [5]St John of God Health Care, Murdoch, Australia, [6]Peter MacCallum Cancer Centre, Melbourne, Australia, [7]Alfred Health, Melbourne, Australia, [8]Monash Health, Clayton, Australia, [9]Sydney Children's Hospital, Randwick, Australia, [10]Townsville Hospital, Townsville, Australia, [11]Princess Alexandra Hospital, Woolloongabba, Australia, [12]Prince of Wales Hospital, Randwick, Australia, [13]Gosford Hostpial, Gosford, Australia, [14]Children's Cancer Centre, Sydney, Australia, [15]McCloud Consulting Group, Sydney, Australia, [16]Royal Prince Alfred Hospital, Camperdown, Australia, [17]Wollongong Hospital, Wollongong, Australia, [18]Westmead Hospital, Westmead, Australia, [19]Canberra Hospital, Garren, Australia, [20]Austin Hospital, Heidleberg, Australia, [21]The Children's Hospital Westmead, Westmead, Australia, [22]Royal North Shore Hospital, St Leonards, Australia, [23]Royal Adelaide Hospital, Adelaide, Australia, [24]Equal contributions, ,

Aim: To assess the impact, on MRD, of consolidation blinatumomab in defined genomic subsets from the ALLG ALL09 (SUBLIME) study and compare this to similar genomic subsets in the predecessor ALLG ALL06 study, which used standard cytotoxic consolidation.

Method: Genomic drivers were subtyped and classified into adverse (AR) vs standard (SR) outcome risk. MRD testing was performed using ASO-PCR (where marker defined).

Results: In the ALL09 cohort 44/55 patients underwent genomic subtyping with 39/44 having MRD available. In the ALL06 cohort 38 B-ALL patients had genomics and MRD available. Genomic subtypes varied between the 2 studies but there were similar proportions of AR (67% in ALL09 vs 62.5% in ALL06).The most frequent subtypes were *PAX5alt* (18%) and *PAX5* p.P80R (11%) in ALL09 versus *KMT2Ar* and *DUX4r* (both 17.5%) in ALL06. The Ph-like subset was 13% in ALL09 and 12.5% in ALL06.

Considering only the AR cohort, MRD positivity reduced from 90% to 70% in ALL06 versus 88% to 36% ($p<0.05$) in ALL09 (Day33/TP1 to Day79/TP2). Despite this overall improvement, not all genomic subtypes identified in ALL09 followed this trend (Table 1).

In ALL09, 15 patients proceeded to High Risk1/2 blocks and sustained MRD positivity was noted in patients harbouring *PAX5 p.P80R (3/3)*, *KMT2Ar* (2/2) and TCF3r (2/3). Of the 9 patients who proceeded to transplant 4 had *PAX5* lesions, 2 were *KMT2Ar* and 2 of the remaining 3 had AR genomic lesions. Relapse was associated with the *TCF3r* subtype in 2 patients (1 died), and the other relapse associated deaths were in the hypodiploid and *PAX5* subsets (both n=1).

Conclusion: These preliminary analyses suggest the addition of blinatumomab in ALL09 significantly decreases the proportion of patients with TP2 MRD positivity, particularly those in the AR cohort compared to ALL06. Genomic analyses also suggests that blinatumomab responsiveness may be associated with specific genomic subtypes. Further follow-up will provide increased clarity.

TABLE 1.	**ALLG-ALL06**		**ALLG-ALL09**	
	# patients	**TP2 positive** (# MRD analysed)	**# patients**	**% TP2 positive** (# MRD analysed)
AR Genomics	25	70% (23)	29	36% (25)
SR Genomics	15	47% (15)	15	43 % (14)
Ph-like (AR)	5	40% (5)	6	17% (6)
***KMT2Ar* (AR)**	7	80%(5)	4	100% (3)
***DUX*4r (AR)**	7	71% (7)	4	66% (3)
PAX5alt (AR)	1	100 % (1)	8	14% (7)
***PAX5* p.P80R (SR)**	3	30% (3)	5	75% (4)
***TCF3r* (SR)**	0		3	100% (3)

Outcomes of prognostically favourable Acute Myeloid Leukaemia(AML) consolidated with chemotherapy only in comparison with allogeneic haematopoietic stem cell transplantation(AlloSCT) in first complete remission(CR1) -single centre experience.

Yea Bing T[1], Ngee Siang L[1], Sen Mui T[1]
[1]*Hospital Ampang, Malaysia, Ampang, Malaysia*

Aim: This was a retrospective analytical study on outcomes of AML with NPM1mut, t(8;21), and inversion(16) or t(16:16) who were consolidated with chemotherapy alone compared with AlloSCT in CR1.

Method: We included a 5-year cohort of patients from 1st January 2011 till 31st December 2015. Kaplan-Meier method was used to assess overall survival. Log-rank test was used to compare survival distributions of 2 cohorts of patients.

Results: 71 patients were analysed. There were 2 cohorts of patients; 1st cohort (71.8%, n=51) of patients consolidated with chemotherapy alone in CR1 and 2nd cohort of patients (28.2%, n=20) consolidated with chemotherapy followed by AlloSCT in CR1.
Overall survival (OS) for 5 years was 42.4%. The median of Progression Free Survival (PFS) time was 13.5 months.
There was no significant difference in survival and PFS among 2 cohorts of patients. The 5-year survival rate for chemotherapy cohort and AlloSCT cohort were 38.5% and 49.4% respectively with P-value 0.722. PFS for chemotherapy cohort was 11.9 months and AlloSCT cohort was 14.2 months with P-value 0.427.
The cause of death in chemotherapy cohort mainly (95%) was due to disease progression and 5% due to neutropenic sepsis. In AlloSCT cohort, 59% of causes of death was due to disease progression, 25% was due to graft-versus-host-disease (GVHD), 8% was due to graft failure and 8% was due to sudden death.

Conclusions: In our study, although the 5-year Survival rate for the AlloSCT cohort was slightly higher by 10%, this was not found to be significant. The reduced benefits in AlloSCT could be contributed by AlloSCT toxicity such as GVHD and infectious complications.

Case Study: rare case of donor cell-derived T-ALL in a female patient after receiving an allo-transplant from her male sibling

Eadie L[1,2], Rehn J[1,2], Schutz C[1], Heatley S[1,2], Kutyna M[1,2], Hiwase D[1,2,3], White D[1,2,4], **Yeung D[1,2,3,4]**
[1]South Australian Health & Medical Research Institute, Adelaide, Australia, [2]University of Adelaide Faculty of Health & Medical Sciences, Adelaide, Australia, [3]Royal Adelaide Hospital, Adelaide, Australia, [4]Australasian Leukaemia & Lymphoma Group , Richmond, Australia

Background: Donor-cell derived leukaemia occurs after stem cell transplant (SCT) at an incidence of ~0.1%. Evidence suggests transfer of pre-leukaemic clones from donor to recipient leading to disease onset in susceptible recipients.

Case Presentation: A 38-year-old female presented with Diffuse Large B-cell Lymphoma in 2011 and received immunochemotherapy (R-CHOP) with high-dose methotrexate as CNS prophylaxis. In 2013, she developed treatment associated acute myeloid leukaemia (t-AML) accompanied by t(8;21). Shet received 7+3 (cytarabine/idarubicin) induction chemotherapy followed by high-dose cytarabine consolidation, prior to a sibling allogeneic SCT in 2014 from her 42-year-old brother. Complete donor chimerism was achieved one month after transplant. In 2018 the patient, then 45-years, presented with cervical lymphadenopathy, leukaemic infiltrates in the lymph nodes and bone marrow, and was diagnosed with T-cell acute lymphoblastic leukaemia (T-ALL). Cytogenetic and genomic analyses identified the leukaemic cells were of male origin and donor-cell derived. Hyper-CVAD therapy lead to a transient response prior to relapse. The patient then received further chemotherapy including nelarabine, before matched unrelated allogeneic SCT. Four months post-second transplant a second relapse in the CNS was confirmed. The patient died fourteen months after T-ALL presentation. The sibling donor is currently well.

Results: Transcriptomic analyses were performed on bone marrow collected at T-ALL presentation and first relapse, and analysed using our bioinformatic pipelines for detection of gene fusions, single nucleotide variants (SNVs), insertion/deletion alterations and assessment of gene expression. Analyses revealed mutations in *KMT2D*, *DNMT3A* and *CNOT3*, two novel *NOTCH1* frameshift mutations, and a novel *STAT5* SNV that potentially contributed to T-ALL relapse. The *STAT5* mutation was activating and targetable with the clinically available drugs, venetoclax and ruxolitinib.

Conclusion: Combined with the patient's history of two additional haematological neoplasms, the T-ALL disease trajectory suggests her bone marrow microenvironment was pre-disposing for leukaemic transformation, in keeping with the 'two-hit' model of ALL development.

NMDP Marrow Harvest Mentorship Program 2023 Review

Cooney J[1]
[1]*Fiona Stanley Hospital, Cottesloe, Australia*

Aim: To review and present up to date expert international information from attendance at the NMDP Marrow Harvest Mentorship Program, which included onsite training and education at Medstar Georgetown University Hospital, Washington DC, USA.

Method: After being selected from widespread international applicants, I was awarded the NMDP mentorship, which included online activities, telephone and teleconferencing via Webex as well as onsite direct involvement in donor selection and screening, harvesting procedures, marrow processing including enumeration of cells and quality as well as sterility, and other measures.

Results: The NMDP Marrow Harvest Mentorship Program provides excellent, comprehensive, up to date information, proposed trials and data collection methods. It promises to provide guidance and excellence regarding all aspects of bone marrow harvesting and cellular processing. Trials involving the use of antibiotics during the donor collection process are being developed, together with other methods to reduce bacterial and other contamination of the graft. The role of enumeration of total nucleated cells (TNCs) and possibly CD34 cells during the harvest to guide collection volumes is also explored and discussed.

Conclusion: As the sixth haematologist selected for the special NMDP Marrow Harvest Mentorship Program, I am pleased to report comprehensive up to date information regarding progress, international experiences, future trials and evidence-based methods to optimise quality products and subsequent outcomes for bone marrow transplant patients. Online resources and contacts will be provided.

HLA Tissue Typing and Haematopoietic stem cell transplant for the Sickle Cell Disease Cohort at the Royal Children's Hospital (Melbourne, Australia): An audit

Duncan A[1], Greenway A[1], O'Reilly E[1], Walker H[1], Clucas L[1]
[1]Clinical Haematology, Royal Children's Hospital, Parkville, Australia, [2]Apheresis Service, Royal Children's Hospital, Parkville, Australia

HLA Tissue Typing and Haematopoietic stem cell transplant for the Sickle Cell Disease Cohort at the Royal Children's Hospital (Melbourne, Australia): An audit

Aim: To determine how many patients from the Sickle cell disease (SCD) population treated at our centre have had human leucocyte antigen (HLA) tissue typing, how many proceeded to Haematopoietic stem cell transplant (HCT) and there outcomes.

Background: Sickle cell disease (SCD) is a serious and potentially life-threatening condition, of which the only established curative treatment is an allogeneic haematopoietic stem cell transplant (HCT). HCT is associated with significant risks, including up to a 10% risk of mortality in SCD[1], and hence careful consideration of a patient's suitability for HCT is required by clinicians.

Method: A retrospective audit was performed on children diagnosed with SCD currently being treated at RCH. Patient's electronic medical records were reviewed to determine if HLA tissue typing was performed, donor availability, HCT details and clinical outcomes.

Results: There are 62 patients with SCD cared for at RCH, 12 of the 62 (19%) have had HLA typing performed. Of these, eight (8/12, 67%) did not have a~~n~~ HLA compatible sibling. Five patients (5/62, 8%) received HCT, four had matched sibling donors. Three of these patients have successfully engrafted, with >96% donor chimerism at 12 months. There was one patient with primary graft failure and one transplant related death.

Conclusion: A small proportion of SCD patients at our centre have had HLA typing performed. For paediatric patients with SCD at RCH, HCT is considered at an individual patient level, based on patient and family factors in conjunction with international guidelines. There is currently limited evidence to determine the age when HCT should be offered, when siblings should be HLA typed, or whether HCT should be considered in the absence of SCD complications. This highlights the need for further research to inform clinical practice for HCT in SCD.

Healthcare resource utilisation and costs associated with allogeneic blood and marrow transplantation: a scoping review

Kim N[1], McErlean G[2], Yu S[1], Kerridge I[3,4,5], Greenwood M[3,4,5], De Abreu Lorenco R[1]
[1]Centre for Health Economics Research and Evaluation, University Of Technology Sydney, Chippendale, Australia, [2]School of Nursing, University of Wollongong, Wollongong, Australia, [3]Northern Clinical School, Faculty of Medicine, University of Sydney, Sydney, Australia, [4]Department of Haematology, Royal North Shore Hospital, St Leonards, Australia, [5]Northern Blood Research Centre, Kolling Institute, University of Sydney, Sydney, Australia

Aim: The objective of this scoping review is to map the evidence regarding healthcare resource utilisation (HRU) and costs associated with allogeneic blood and marrow transplant (allo-BMT).

Method: The scoping review was conducted in accordance with the Joanne Briggs Institute (JBI) methodology for scoping reviews. Electronic databases PubMed, EMBASE and Health Business Elite databases were searched in addition to the grey literature. The databases were searched from inception until November 2022. Any studies that reported HRU and/or costs associated with adult (≥ 18 years) allo-BMT population were eligible for inclusion. Two reviewers independently screened 20% of the sample at each of the two stages of screening (abstract and full text). Monetary values were standardised to 2023 United States Dollars (USD).

Results: Forty-four studies were identified that reported HRU and costs of allo-BMT and were not limited to a particular allo-BMT-associated outcome or adverse event, eg GVHD or infection. The proportion of studies that reported costs and HRU was 93.2% and 79.5%, respectively. Cost calculations and HRU metrics, including the timeframe for which they were reported were heterogeneous across the studies. Length of hospital stay (LOS) was the most commonly reported HRU metric (33 (75%) of studies), with LOS ranging from a median initial hospitalisation of 10 days (reduced intensity chemotherapy; RIC) to 73 days (myeloablative chemotherapy; MAC). The total cost of an allo-BMT ranged from $69,096 (RIC) to $472,318 (MAC) at 100 days, $113,517 (RIC) to $697,625 (allo-BMT with complications) at 1 year, with one study reporting costs of allo-BMT at 5-years of $228,564.

Conclusion: Allo-BMT is a highly cost and resource intense therapy. Policy-makers and government need to be aware not only of the short-term but also the long-term health resource requirements for this patient population. Further research is needed to understand the key determinants of HRU and costs associated with allo-BMT in order to better inform the design and delivery of health care for BMT recipients and ensure the quality, safety and efficiency of care.

Comprehensive Evaluation of Immune Microenvironment in Poor Graft Function following Allogeneic Stem Cell Transplantation

Prabahran A[1,2,3], Wu Z[5], Gao S[5], Morgan H[2], Holzwart N[2], Feng X[5], Rawicki M[2], Wilson C[4], Blombery P[4], Chee L[1,2,3], Ritchie D[1,2,3], Young N[5], Koldej R[2,3]

[1]Department of Clinical Haematology, Peter MacCallum Cancer Centre/ Royal Melbourne Hospital, Parkville, Australia, [2]ACRF Laboratory, The Royal Melbourne Hospital , Parkville, Australia , [3]The University of Melbourne, Parkville, Australia, [4]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia, [5]Hematology Branch, National Heart Lung and Blood Institute, Bethesda, United States of America

Aim:

- Poor Graft Function (PGF) defined by multilineage cytopenias in the setting of complete donor chimerism following allogeneic stem cell transplantation (alloSCT) is associated with Graft versus Host Disease (GVHD), non-CMV viral reactivation and ICU admission in 1st 30 days of alloSCT, suggesting an immunologic basis to the syndrome.

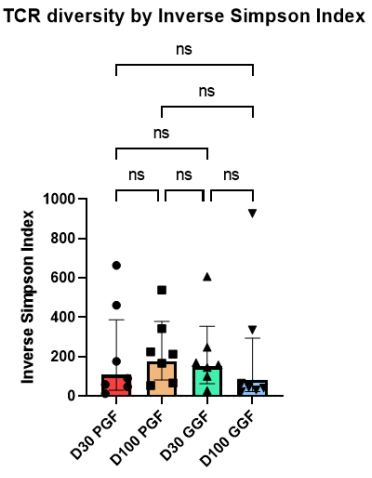
1. TCR sequencing, flow cytometry and single cell RNA sequencing (scRNA-seq) was used to comprehensively evaluate the cellular basis of PGF compared to patients with Good Graft Function (GGF) and Healthy Donors (HD).

Method: Peripheral blood and bone marrow mononuclear cells (PBMCs, BMMCs) and trephine samples were collected from patients as part of a prospective observational study. Flow cytometry was performed on PBMCs and scRNA-seq was performed on the BMMCs. Additionally, TCR sequencing was performed on CD3+ selected chimerism samples at D30 and D100.

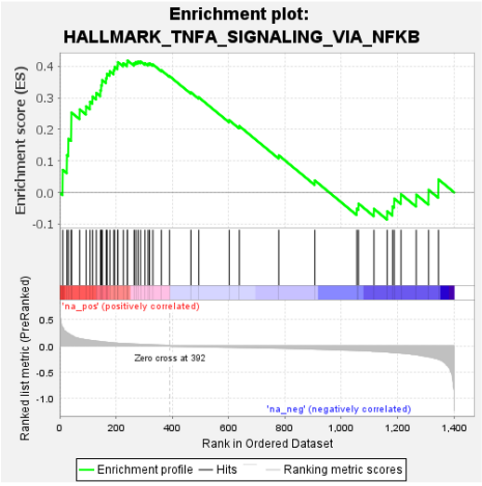
Results: Twenty-four PGF and 23 patients with GGF were analysed. Flow cytometry and sc-RNA seq demonstrated minimal differences in the proportion of T-,B,NK, CD34+ and myeloid subsets between PGF and GGF. Similarly, there were no differences in TCR diversity by inverse Simpson index at D30 and D100 between groups (Figure 1A). Both PGF and GGF demonstrated lower TCR diversity suggestive of oligoclonality compared to HD. There was marked upregulation in inflammatory pathways such as TNF-α signalling by sc-RNA seq, predominantly driven by monocyte/dendritic cells and CD4 T-cells in PGF compared to GGF (Figure 1B).

Conclusion: The similarities of immune cell subsets and restricted TCR diversity between PGF and GGF suggests that an environment for dysregulated T-cell immunity is primed in the post alloSCT setting and is triggered by further inflammatory stimuli such as GVHD and viral infection, leading further immune activation and subsequent suppression of haematopoiesis and bone marrow failure.

A



B



The role of off-study anti-viral cytotoxic T lymphocytes

Tan S[1], Koo R[1,2], Yong M[4,5,6], Rivalland A[1], Hockridge B[1], Mcpherson E[1], Ritchie D[1,2,3]
[1]Department of Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Parkville, Australia, [2]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, Australia, [3]Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia, [4]Department of Infectious Diseases, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Parkville, Australia, [5]The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital, Parkville, Australia, [6]National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Parkville, Australia

Background: Cytotoxic T lymphocytes (CTL) have an emerging role in viral reactivation following allogeneic stem cell transplantation (alloSCT). For patients in our service who cannot access CTL via trial participation, we utilise a special access scheme (SAS) supply program that is reviewed and approved by our Drug and Therapeutics Committee (DTC).

Method: Since 2016, 27 patients within Royal Melbourne Hospital (RMH) and Peter MacCallum Cancer Centre (PMCC) have received CTL, of which 14 received CTL through the SAS program. Only SAS product recipients (n=9) of cytomegalovirus (CMV) directed CTL are reported here.

Results: The indication for off-study CTL included persistent CMV viraemia (n=7, 78%), CMV disease (n=2, 22%), proven or anticipated intolerance of antiviral therapy such as poor graft function (n=8, 89%), concurrent GVHD requiring heavy immunosuppression (n=6, 67%) or viral recurrence (n=3, 33%).

Four (44%) patients received ATG during conditioning. Two out of nine CMV-seropositive recipients received transplants from CMV-seronegative donors; D-/R+ (n=2, 22%) or D+/R+ (n=7, 78%). Three (33%) patients received only 1 infusion, whereas six (67%) patients received 3 or more infusions. Median time to first infusion was 195.5 days (range 75 to 1661) post-day zero.

Of the 9 treated patients, 2 had no response, 6 had a complete response and 1 had incomplete viraemic control. Of those who achieved a response (n=7), 4 patients remain alive (1-year OS: 60%). Causes of death include progressive disease, GVHD and sepsis (n=1, respectively). Of those who had a complete response (n=6), two patients had second reactivation post CTL requiring anti-viral therapy. None of the patients had infusion related complications.

Conclusion: A prospective SAS mediated access CTLs program is a meaningful adjunct to patients with anti-viral intolerance or recurrent CMV infection post alloSCT, outside of clinical trials.

Successful stem cell collection and BCNU-T ASCT despite previous BEAM ASCT in a patient with multi-relapsed mantle cell lymphoma.

Tong T[1], McKeough N[1], Cochrane T[1], Toland P[1], Barr J[1], Ng T[1]
[1]*Gold Coast University Hospital, Southport, Australia*

Case: A 62-year-old male presented with right monocular vision loss with a pituitary lesion from central nervous system (CNS) progression of mantle cell lymphoma (MCL) in November 2022 following diagnosis and myeloablative treatment in July-December 2020. Initial complete remission (CR) was achieved with NORDIC MCL2 protocol consisting of 3 cycles R-MaxiCHOP/HiDAC and consolidated with BEAM autologous stem cell transplant (ASCT) in December 2020. 6.13x10^6/kg (ABW) CD34+ cells were harvested with all cells reinfused.

CNS relapse occurred less than 12 months post ASCT in September 2021 with an intradural lesion treated with Ibrutinib and intrathecal (IT) chemotherapy to achieve CR. Further CNS relapse in November 2022 was managed with IT chemotherapy (hydrocortisone, methotrexate, cytarabine) and 3 cycles of R-MATRIX and 1 cycle of RICE per the MARIETTA protocol.

Stem cell mobilisation commenced off cycle 3 R-MATRIX (February 2023) utilising 10µg/kg granulocyte colony stimulating factor (G-CSF) with collection occurring on days 15-17 requiring plerixafor rescue (0.24mg/kg day 2 and 3 of collection respectively) to yield 3.67x10^6/kg (ABW) fresh CD34+ with an average cell recovery post cryopreservation of 83%. Peripheral blood counts and harvest yields are outlined in the table below.

Pre collect white cell count (x 10^9/L) - pre CD34 (/µL)	Fresh CD34 (ABW) (x10^6/kg)	Thaw CD34 (ABW) (x10^6/kg)	Recovery	Viability
8.4 / 5.84	0.76	0.68	91%	91%
18.7 / 13.72	1.56	1.21	77%	78%
23.2 / 11.88	1.35	1.12	83%	89%

(Definitions: BCNU-T/ carmustine-thiotepa); BEAM/ carmustine-cytarabine-etoposide-melphalan; R-MaxiCHOP/ rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone; HiDAC/ high dose cytarabine; MATRIX/ methotrexate-cytarabine-thiotepa-rituximab; RICE/ rituximab-ifosphamide-carboplatin-etoposide; ANC/ absolute neutrophil count; ABW/ actual body weight).

Lumbar puncture after MARIETTA completion was negative for disease prior to BCNU-T autograft on 18th April 2023. Subsequently, reinfusion was performed over two days due to large dimethyl sulfoxide (DMSO) dose. Neutrophil engraftment occurred on Day 10 (defined as absolute neutrophil count >0.5x10^9/L) and platelet engraftment occurred on Day 17 (defined as platelet >20x10^9/L unsupported).

Discussion: This case demonstrates that recollection of haemopoietic stem cells is still achievable even after previous myeloablative therapy with stem cell rescue. In addition, the MARIETTA approach is a feasible treatment option in CNS relapse of MCL which carries a devastating prognosis.

"Small but mighty": A case report and proposed guide to peripheral blood stem cell collection in an infant

Walker H[1], Metz D[1], Greenway A[1], Fleming J[1], O'Reilly E[1], Millard Z[1], Cusack B[1], Duncan A[1], Clucas L[1]

[1]The Royal Children's Hopsital, Melbourne, Australia, [2]Murdoch Children's Research Institute, Melbourne, Australia, [3]The University of Melbourne, Melbourne, Australia

Aim: This case report describes the challenges and unique considerations related to performing apheresis in a small infant to facilitate stem cell collection. This small infant collection was challenging for our team and the aim of this case report is to provide a guide to other centres of what are the unique considerations to achieve a successful stem cell collection in infants < 10kg.

Method: This case describes our experience at The Royal Children's Hospital of stem cell collection for a 5kg infant who required stem cell supported chemotherapy as part of treatment for a brain tumour. Continuous mononuclear cell collection (cMNC) protocol was applied using the Terumo device.

Results: This case study provides a summary for key considerations and recommendations for apheresis in a small infant; including location of procedure, vascular access, monitoring, anticoagulation, calcium monitoring and inlet flow rates. This included performing the procedure in the intensive care unit to facilitate required monitoring frequency, electing to run a continuous calcium gluconate infusion to prevent hypocalcaemia and using Acid citrate dextrose (ACD) anticoagulation.

Conclusion: There is limited evidence for the ideal method for peripheral stem cell collection in small infants < 10kg. This population of patients are technically complex due to lower total body volume, smaller blood vessel size and increased vulnerability to the metabolic and haemodynamic changes that occur due to apheresis. Successful peripheral stem cell collection using cMNC will become increasingly important as a tool for autologous stem cell transplant and future therapies such as gene therapy. The learnings from this patient described has led to a change in local practice to optimise stem collection in this population of small infants.

An unusual case of chronic lymphocytic leukaemia with Richter transformation in a young adult

Chatterjee S[1], Squires R[1]
[1]*Gold Coast University Hospital, Gold Coast, Australia*

Development of aggressive lymphoma on a background of chronic lymphocytic leukaemia (CLL), known as Richter transformation (RT), occurs in 2-9% of cases, with a median time from diagnosis of CLL to transformation ranging from 2-5 years [1].

A 23-year-old female presenting with a year-long history of dyspnoea and cough, without any B-symptoms, was found to have a large mediastinal mass on chest X-ray. She was noted to have a marked leucocytosis of 94.3 x 10/L with predominantly small mature lymphocytes on blood film and a LDH of 392 U/L. Bone marrow biopsy demonstrated heavy infiltration with a mature lymphoid population constituting 82% of the total cells, consistent with CLL on flow cytometry and histology. No genetic aberrations were found in *TP53* and microarray was normal. An 18^{F}fluorodeoxyglucose (FDG) positron emission tomography (PET) scan revealed FDG-avidity of the mediastinal mass, as well as widespread nodal disease and splenic involvement. Histology of a core biopsy from the mediastinal mass demonstrated an abnormal large lymphoid population, with features suggestive of a diagnosis of RT. Immunohistochemistry on this population was positive for CD5, CD19, CD20, and BCL-2 and negative for CD10, CD23, CCND1, and BCL-6. Ki67 proliferation index was 30-40%. *IgHV* was unmutated in both tissue and bone marrow, with a matching productive clonal rearrangement. The patient was initiated on venetoclax, dose-adjusted rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin (VR-EPOCH), and is undergoing planning for allogenic haematopoietic cell transplantation (alloHCT). Interim PET scan after cycle 3 demonstrated a complete metabolic response and near complete anatomic response.

Our case offers a longitudinal insight into the treatment and outcomes of a young patient with RT, typically studied in older cohorts (median age of CLL diagnosis is 70). Young age (<55) is associated with higher risk of RT [2]. Unmutated *IgHV* is a common feature of RT arising from CLL, conferring increased risk of progressive disease and poor prognosis [3]. Our treatment strategy is based on a recent single-arm, phase 2 trial of VR-EPOCH in which 13 out of 26 patients achieved complete remission, with a durable response (overall survival of 19.6 months) compared to existing regimens [4]. Long-term remission is reported post alloHCT [5].

[1] Eyre TA, Riches JC, Patten PEM, Walewska R, Marr H, Follows G, et al. Richter transformation of chronic lymphocytic leukaemia: a British Society for Haematology good practice paper. Br J Haematol. 2022;196:864-70.
[2] Mauro FR, Foa R, Giannarelli D, Cordone I, Crescenzi S, Pescarmona E, et al. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. Blood. 1999 Jul 15;94(2):448-54.
[3] Mao Z, Quintanilla-Martinez L, Raffeld M, Richter M, Krugmann J, Burek C, et al. IgVH mutational status and clonality analysis of Richter's transformation: diffuse large B-cell lymphoma and Hodgkin lymphoma in association with B-cell chronic lymphocytic leukemia (B-CLL) represent 2 different pathways of disease evolution. Am J Surg Pathol. 2007 Oct;31(10):1605-14.
[4] Davids MS, Rogers KA, Tyekucheva S, Wang Z, Pazienza S, Renner SK, et al. Venetoclax plus dose-adjusted R-EPOCH for Richter syndrome. Blood. 2022 Feb 3;139(5):686-89.
[5] Kharfan-Dabaja MA, Kumar A, Hamadani M, Stilgenbauer S, Ghia P, Anasetti C, et al. Clinical practice recommendations for use of allogeneic hematopoietic cell transplantation in chronic lymphocytic leukemia on behalf of the guidelines committee of the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2016 Dec;22(12):2117-2125.

A phase 1 study with the novel B-cell lymphoma 2 (Bcl-2) inhibitor BGB-11417 as monotherapy or in combination with zanubrutinib in patients with CLL/SLL: preliminary data

Cheah C[1,2,3], Tam C[4,5], Lasica M[6], Verner E[7,8], Browett P[9], Anderson M[10,11], Hilger J[12], Fang Y[12], Simpson D[12], Opat S[6,13]

[1]Department of Haematology, Sir Charles Gairdner Hospital and PathWest Laboratory Medicine, Nedlands, Australia, [2]Medical School, University of Western Australia, Crawley, Australia, [3]Linear Clinical Research, Nedlands, Australia, [4]Alfred Hospital, Melbourne, Australia, [5]Monash University, Clayton, Australia, [6]St Vincent's Hospital Melbourne, Fitzroy, Australia, [7]Concord Repatriation General Hospital, Concord, Australia, [8]University of Sydney, Sydney, Australia, [9]Department of Haematology, Auckland City Hospital, Auckland, New Zealand, [10]Peter MacCallum Cancer Centre, Melbourne, Australia, [11]Division of Blood Cells and Blood Cancer, The Walter and Eliza Hall Institute, Parkville, Australia, [12]BeiGene (Shanghai) Co., Ltd., BeiGene USA, Inc., Shanghai/San Mateo, China and USA, [13]Monash Health, Clayton, Australia

Aim: BGB-11417-101 (NCT04277637), an ongoing, first-in-human, phase 1/1b dose-escalation/expansion study, assessed BGB-11417 (a highly selective Bcl-2 inhibitor) as monotherapy or in combination with zanubrutinib, a next-generation Bruton tyrosine kinase inhibitor. CLL/SLL cohort data are presented.

Method: Patients received BGB-11417 (40mg/80mg/160mg/320mg or 640mg once daily [QD]) with dose ramp-up to mitigate tumor lysis syndrome (TLS). In combination cohorts, patients received zanubrutinib (320mg QD or 160mg twice daily) 8-12 weeks before BGB-11417. A Bayesian logistic regression model evaluated dose-limiting toxicity during dose ramp-up through day 21. Minimal residual disease (MRD) was assessed per European Research Initiative on CLL flow cytometry assay.

Results: By 15May2022, 50 patients with CLL received treatment: n=6 monotherapy (all relapsed/refractory [R/R]) and n=44 combination (R/R, n=22; treatment naïve [TN], n=22). The monotherapy cohort received BGB-11417 doses ≤160mg; combination cohorts received doses ≤640mg (R/R) or ≤320mg (TN; n=8 in zanubrutinib pretreatment not yet dosed with BGB-11417). With dose escalation ongoing, no cohort reached maximum tolerated dose. Median follow-up was 11.5 months (range 8.5-18.3; monotherapy) and 5.8 months (range 0.2-10.5; combination). With monotherapy, cytopenias were the most common treatment-emergent AEs (TEAEs; ≥50%; grade ≥3, 33%). With combination treatment, contusion, neutropenia, and low-grade gastrointestinal toxicity were most common (≥23%); neutropenia was the most common grade ≥3 TEAE (11%). One patient discontinued combination treatment (disease progression; Richter transformation); none discontinued monotherapy. One patient (monotherapy) had laboratory TLS (overall ≤2%) that resolved without intervention. No clinical TLS occurred. Most patients had reduced absolute lymphocyte counts with responses seen with ≥1mg. Among 4 MRD-evaluable patients (160mg), 3 (n=2 monotherapy, n=1 combination) had peripheral blood CLL counts <10-4 at 24 weeks after BGB-11417 initiation.

Conclusion: Preliminary data show that BGB-11417 ± zanubrutinib was well tolerated. Grade ≥3 neutropenia was uncommon and manageable; TLS rates were low. Efficacy was supported by rapid ALC reduction during ramp-up.

Lisaftoclax (APG-2575) safety and activity as monotherapy or combined with acalabrutinib or rituximab in treatment-naïve, relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma (R/R CLL/SLL): initial phase 2 global study data

Davids M[1], Chanan-Khan A[2], Mudenda B[3], Nogaieva L[4], Kriachock I[5], Usenko H[6], Ivanov V[7], Kyselova O[8], Perekhrestenko T[9], Muzhychuk I[10], Myasnikov A[11], Lukavetskyy L[12], Proydakov A[13], Uspenskaya O[14], Borisenkova E[15], Marlton P[16], Siddiqi T[17], Winter A[18], Lysa T[19], Bakirov B[20], Fu L[3], Chen Z[21], Yu M[21], Li M[3], Glass L[3], Ahmad M[3], Karpenko O[8], Osipov I[7], De A[3], Paudyal B[3], Wang H[21], Winkler R[3], He Z[22], Chen J[22], Gabrail N[23], Ganju V[24], Konstantinova T[25], Samoilova O[26], Yang D[3,21], Zhai Y[3,21]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, US, [2]Mayo Clinic, Jacksonville, US, [3]Ascentage Pharma Group Inc., Rockville, US, [4]MI "Cherkasy ROD" of Cherkasy Regional Council, Regional Treatment and Diagnostic Hematology Center, Cherkasy, UA, [5]Oncohematology, National Cancer Institute, Ukraine Hemoblastoses of Division of Conservative Methods of Treatment, Kyiv, UA, [6]Dnipropetrovsk Regional Hematology Center, Dnipro, UA, [7]Almazov National Medical Research Center, St. Petersburg, RU, [8]Medical Center "Ok!Clinic+", Kyiv, UA, [9]Medical Center Named by Academician Yuriy Spizhenko (ARENSIA Exploratory Medicine Unit), Kyiv, UA, [10]SI SP Grygoriev Institute of Radiology of the NAoMS of Ukraine, Kharkiv, UA, [11]Republican Hospital n.a. V.A. Baranov, St. Petersburg, RU, [12]SI "Institute of Blood Pathology and Transfusion Medicine of NAoMS of Ukraine," Hematology Department, Lviv, UA, [13]State Institution Komi, Krasnozatonsky, RU, [14]Leningrad Regional Clinical Hospital, St. Petersburg , RU, [15]Kaluga Regional Clinical Hospital, Kaluga, RU, [16]Princess Alexandria Hospital and University of Queensland, Brisbane, AU, [17]City of Hope, Duarte, US, [18]Cleveland Clinic, Cleveland, US, [19]Regional Clinical Hospital n. a. O. F. Herbachevskyi, Zhytomyr, UA, [20]Bashkir State Medical University, Ufa, RU, [21]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, CN, [22]Ascentage Pharma Pty Ltd., Sydney, AU, [23]Gabrail Cancer Center, Canton, US, [24]PASO Medical, Frankston, AUS, [25]Regional Clinical Hospital No1 , Ekaterinburg, RU, [26]Nizhny Novgorod Regional Clinical Hospital named after N A Semashko, Nizhny Novgorod , RU

Aim: Investigational BCL-2 inhibitor lisaftoclax is active in patients with CLL/SLL, including those with high-risk factors or progressive disease following Bruton tyrosine kinase inhibitor (BTKi) treatment. We report safety and efficacy data of lisaftoclax alone or combined with acalabrutinib or rituximab in these patients.

Method: Lisaftoclax was administered as daily ramp-up at 20, 50,100, 200, and up to 400 (n = 47), 600 (76), and 800 (41) mg, depending on the target dose. Primary objectives were to determine RP2D, safety, and efficacy, including overall response rates (ORRs) in each treatment cohort.

Results: As of December 5, 2022, 164 patients (148 R/R; 16 treatment-naïve) enrolled from 28 sites in the United States, Australia, and Europe (median [range] age, 62 [18-80] years) were treated with lisaftoclax (n = 46), lisaftoclax + rituximab (39), or lisaftoclax + acalabrutinib (79). Median (range) treatment durations per cohort were 16.5 (1-36), 11 (0-21), and 11 (1-24) cycles, respectively. The lisaftoclax + acalabrutinib group included patients with treatment-naïve (n = 16), venetoclax-resistant (6), and BTKi-refractory (7) disease. Any-grade (>5%) adverse events in all cohorts included neutropenia (30% [26% grade 3/4]); COVID-19 infection (26%); anemia (24% [12% grade 3/4]); diarrhea (20%); thrombocytopenia (17% [5% grade 3/4]); hyperuricemia or pyrexia (9% each); nausea, headache, or fatigue (8% each); increased AST (7%); hyperphosphatemia (6%); and increased creatinine (6%). Tumor lysis syndrome (TLS) was observed in 4 (2.5%) patients (2 clinical/2 laboratory). ORRs were 65%, 87%, and 98% in the monotherapy, lisaftoclax + rituximab, and lisaftoclax + acalabrutinib cohorts, respectively.

Conclusion: A 1-week dose ramp-up with lisaftoclax was feasible, with a low incidence of biochemical TLS with no clinical sequelae observed. Chronic dosing with lisaftoclax alone or combined with rituximab or acalabrutinib had a manageable safety profile and preliminary efficacy was synergistic in the rituximab and acalabrutinib combination groups (APG2575CU101; NCT04215809).

Incidence, prevalence, and mortality of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in Australia

Gao L[1], Cheah C[2,3], Nguyen D[1], Bohingamu Mudiyanselage S[1], Tang B[4], Zhao F[5]
[1]Deakin Health Economics, Institute for Health Transformation, School of Health & Social Development, Faculty of Health, Deakin University, Melbourne, Australia, [2]Department of Haematology, Sir Charles Gairdner Hospital, Perth, Australia, [3]Medical School, University of Western Australia, Perth, Australia, [4]BeiGene USA, Inc, San Mateo, USA, [5]BeiGene AUS PTY Ltd, Sydney, Australia

Aim: In Western countries, CLL/SLL is the most common leukemia; however, epidemiological data in Australia are limited. We aimed to quantify the incidence, prevalence, and mortality rate for CLL/SLL and predict 30-year incidence and prevalence trends in Australia.

Method: All CLL/SLL cases from Jan 2009 through Dec 2018 from 4 states (Victoria, Tasmania, ACT, and Queensland) were identified in the Australian cancer database using *International Statistical Classification of Diseases* (*ICD-10-AM* code C83.0/C91.1, histology code 9823). Incidence, prevalence, and mortality rates were calculated using DisMod II and Australian Institute for Health and Welfare approaches. Thirty-year incidence and prevalence rate predictions were modeled using least-squares linear regression. A Kaplan-Meier estimator was constructed for survival analysis. All analyses were stratified by sex, age group, and diagnostic year, as applicable.

Results: The crude annual incidence of CLL/SLL (58% in the 60- to 79-year age group) was 834-1278 and 479-786 per 10,000,000 person-years for males and females, respectively, with an increase in 10-year crude rates (male: coefficient, 44.83; *P*<.0001; female: coefficient, 26.91; *P*=.014). Age-standardized incidence rates were 600-888 per 10,000,000 person-years. The observed prevalence rate was highest in the ≥80-year age group (range, 5001-8654 and 2459-4656 per 10,000,000 persons for males and females, respectively) and lower in the 40- to 49-year age group (range, 628-1041 and 314-470 per 10,000,000 persons, respectively). An increase in prevalence was predicted (male: coefficient, 647.06; *P*<.001; female: coefficient, 381.97; *P*<.0001). The crude annual CLL/SLL mortality rate was 83-295 per 10,000,000 persons, with the 2017 rate being highest. Over 53% of patients were alive at 10 years of follow-up, with no difference observed between sexes (*P*=.0608). Patients diagnosed after 2015 had better survival than those in earlier years (*P*=.038).

Conclusion: Incidence and prevalence of CLL in Australia have been increasing over the last decade, while survival has improved in recent years.

Treatment preferences and goals of people living with Chronic Lymphocytic Leukaemia

Fifer S, **Godsell J**[1], Opat S, Hamad N, Lasica M, Forsyth C, Morand L, Smeaton E, Winton S, **Puig A**[1], McGeachie M
[1]*Janssen, Macquarie Park, Australia*

Chronic lymphocytic leukaemia: the patient experience in Australia

Aim: This research aimed to explore the patient experience across the healthcare pathway among people living with Chronic Lymphocytic Leukaemia (CLL) in order to help identify potential areas that could be improved.

Method: Eleven factors or 'moments that matter' (MTM), had been previously identified via qualitative research. Each MTM describing a different aspect of the patient journey was integrated into a 30-to-40-minute online survey that incorporated Best Worst Scaling (BWS) methodology, a technique that takes advantage of an individual's ability to reliably identify extremes ('best' and 'worst') in sets of items. This study implemented a novel anchoring process to rescale best-worst scores for importance and satisfaction which could be combined to form a CLL Patient Experience Index (PEI). Additional survey questions were included to help identify potential ways to improve patient satisfaction.

Results: 25 CLL patients with treatment experience completed the BWS experiment. The median PEI score (i.e., overall satisfaction score) was 66.2 (out of a possible 100). The top four MTM that were most important to patients, but they were least satisfied with, were access to/effectiveness of medication (52%), support for their support person (48%), access to other treatment/support services (48%) and monitoring/identifying progress/deterioration (44%).

Conclusion: Findings from this research provide insight into the experiences and needs of CLL patients within the Australian healthcare system and can help inform decisions that would improve patient care and treatment experience. As expected, the results indicate that patients most value ability to access effective medication and support services to manage their CLL. This research also highlights other aspects of patient-centric care, such as support for the patient's support person, which, may be overlooked along the patient journey. This research highlights that even small adjustments have the potential to improve the patients' experience within the healthcare system.

Chronic Lymphocytic Leukaemia: The Patient Experience in Australia

Fifer S, **Godsell J**[1], Opat S, Hamad N, Lasica M, Forsyth C, Morand L, Smeaton E, Puig A[1], McGeachie M, Winton S

[1]*Janssen, Macquarie Park, Australia*

Aim: This research aimed to explore the patient experience across the healthcare pathway among people living with Chronic Lymphocytic Leukaemia (CLL) in order to help identify potential areas that could be improved.

Method: Eleven factors or 'moments that matter' (MTM), had been previously identified via qualitative research. Each MTM describing a different aspect of the patient journey was integrated into a 30-to-40-minute online survey that incorporated Best Worst Scaling (BWS) methodology, a technique that takes advantage of an individual's ability to reliably identify extremes ('best' and 'worst') in sets of items. This study implemented a novel anchoring process to rescale best-worst scores for importance and satisfaction which could be combined to form a CLL Patient Experience Index (PEI). Additional survey questions were included to help identify potential ways to improve patient satisfaction.

Results: 25 CLL patients with treatment experience completed the BWS experiment. The median PEI score (i.e., overall satisfaction score) was 66.2 (out of a possible 100). The top four MTM that were most important to patients, but they were least satisfied with, were access to/effectiveness of medication (52%), support for their support person (48%), access to other treatment/support services (48%) and monitoring/identifying progress/deterioration (44%).

Conclusion: Findings from this research provide insight into the experiences and needs of CLL patients within the Australian healthcare system, and can help inform decisions that would improve patient care and treatment experience. As expected, the results indicate that patients most value ability to access effective medication and support services to manage their CLL. This research also highlights other aspects of patient-centric care, such as support for the patient's support person, which, may be overlooked along the patient journey. This research highlights that even small

A matching-adjusted indirect comparison (MAIC) of the efficacy and safety of acalabrutinib (acala) versus zanubrutinib (zanu) in relapsed or refractory chronic lymphocytic leukemia (RR CLL)

Kittai A[1], Skarbnik A[2], Miranda M[3], SM Yong A[4], Roos J[4], Hettle R[3], Palazuelos-Munoz S[4], Shetty V[4], **Kavanagh D[1]**, Ghia P[6]

[1]Division of Hematology, The Ohio State University Comprehensive Cancer Center, Columbus, USA, [2]Novant Health Cancer Institute, Lymphoma and CLL program, Charlotte, USA, [3]AstraZeneca, Cambridge Biomedical Campus, UK, [4]AstraZeneca, Wilmington, USA, [5]AstraZeneca, Sydney, Australia, [6]Università Vita-Salute San Raffaele, Milan, Italy

Aim: The BTKi ibrutinib was compared head-to-head with second-generation BTKi's in ELEVATE-RR (acala) and ALPINE (zanu). However, study population differences prevented comparison of acala and zanu. Acala was also assessed in ASCEND, which had a similar population to ALPINE but a different comparator. Thus, we used an unanchored MAIC to compare the efficacy and safety of acala vs zanu.

Methods: In the unanchored MAIC, acala individual patient data (IPD) from ASCEND were weighted to match zanu baseline data from ALPINE. An efficacy analysis assessed investigator-assessed PFS (INV PFS) in randomized patients with baseline data (acala, n = 149; zanu, n = 327). Pseudo
IPD for INV PFS for zanu were obtained from Kaplan-Meier curves. A safety analysis assessed odds ratios (ORs) of AEs in treated patients with baseline data (acala, n = 148; zanu, n = 324). To allow comparison of the incidence of AEs, an artificial data cut-off (Feb 21, 2020) was imposed for acala to match the zanu median treatment exposure (both 28.4 months).

Results: After matching, the effective sample size of acala was 99 (66.6%; 65% male; median age 66 years). 12- and 24-month INV PFS are shown below. The MAIC hazard ratio (HR) for INV PFS is equivalent for acala vs zanu (HR 0.90). The risk of having grade ≥ 3 AE (OR 0.66), atrial fibrillation (AF; OR 1.32), grade ≥ 3 AF/atrial flutter (OR 0.60), grade ≥ 3 hemorrhage (OR 0.61) or an AE leading to discontinuation (OR 1.14) was similar with acala vs zanu. The risk of having a serious AE (OR 0.61), hypertension (any grade: OR 0.18; grade ≥ 3: OR 0.22), any grade hemorrhage (OR 0.54) or an AE leading to dose reduction (OR 0.30) was lower with acala vs zanu.

Conclusions: Acala and zanu have similar efficacy in patients with RR CLL, while acala has a lower risk of grade ≥ 3 hemorrhage, any grade and grade ≥ 3 hypertension and dose reduction due to AEs vs zanu. Limitations of MAIC analyses mean the results should be viewed as hypothesis-generating.

Landmark INV PFS Treatment	**12-month INV PFS % (95% CI)**	**24-month INV PFS** % (95% CI)
Acala pre-matching	89 (83–93)	75 (68–81)
Acala post-matching	91 (84–95)	76 (66–84)
Zanu	92 (88–94)	78 (73–83)

Cumulative review of heart failure with acalabrutinib in the treatment of chronic lymphocytic leukemia using data from clinical trials and post-marketing experience

Ghia P[1], Pareek M[2], Apkarian Bourjlian S[3], Bajwa N[3], J. Corry A[3], Jannuru S[4], Kreuzbauer G[5], **Kavanagh D[6]**, Abdel-Qadir H[7]
[1]Università Vita-Salute San Raffaele and IRCCS Ospedale, San Raffaele, Italy, [2]Center for Translational Cardiology and Pragmatic Randomized Trials, Copenhagen University Hospital – Herlev and Gentofte,, , Denmark, [3]AstraZeneca, Gaithersburg, USA, [4]AstraZeneca, South San Francisco, USA, [5]AstraZeneca, Baar,, Switzerland, [6]AstraZeneca, Sydney, Australia, [7]Women's College Research Institute, Women's College Hospital, Toronto, Canada

Aim: To review the acalabrutinib safety profile with respect to HF based on phase 3 randomized clinical trial data and the global acalabrutinib safety database.

Methods: Safety data were obtained from 3 phase 3 CLL trials (ELEVATE-RR, ELEVATE-TN, ASCEND) and from the global acalabrutinib safety database, which includes all clinical trial and post-marketing data for acalabrutinib. In the individual phase 3 clinical trials for the acalabrutinib and active comparator arms, exposure-adjusted (exp-adj) incidence rates (events/100 person-months) were reported for "cardiac failure" (CF) using the broad Standardized MedDRA Query (SMQ; comprising CF-specific and CF-related terms) and the MedDRA preferred terms (PT). Exp-adj incidence rates of CF also were reported based on a cumulative comprehensive search of the global acalabrutinib safety database.

Results: In the 3 clinical trials, 598 patients were treated with acalabrutinib monotherapy, 178 with acalabrutinib + obinutuzumab, and 586 with comparator anti-neoplastic agents. In each of the 3 clinical trials, the overall exp-adj incidence rates of any-grade and grade ≥3 CF (SMQ) were numerically lower in the acalabrutinib arms vs comparator arms; the most common PTs were generally numerically lower in the acalabrutinib arms (**Table**). The exp-adj incidence rate of any-grade CF (PT) in the acalabrutinib arms across the 3 trials ranged from 0.03 to 0.06 (**Table**); the corresponding exp-adj incidence rate of any-grade CF from the global acalabrutinib safety database (post-marketing sources only) was 0.008. In the global acalabrutinib safety database, a total of 779 CF (SMQ) events were captured (727 from post-marketing sources), of which the most commonly reported CF terms were peripheral swelling (n=406), edema peripheral (n=99), edema (n=56), and pulmonary edema (n=47).

Conclusions: Cumulatively, overall exp-adj incidence estimates for CF events from 3 clinical trials in CLL were not higher with acalabrutinib monotherapy than with other antineoplastic agents, irrespective of severity. Data from the clinical trial and global safety databases do not suggest that acalabrutinib is associated with a substantially higher risk of CF.

BRUIN CLL-314: A phase 3, open-label, randomized study of pirtobrutinib versus ibrutinib in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (trial in progress)

Lewis K[1], Woyach J[2], Coombs C[3], Escalón M[4], Ceccarelli M[4], Bian Y[4], Wierda W[5]
[1]Sir Charles Gairdner Hospital And Linear Clinical Research, Nedlands, WA 6009, Australia, [2]The Ohio State University Comprehensive Cancer Center, , Columbus, USA, [3]University of California Irvine, Irvine, USA, [4]Eli Lilly and Company, Indianapolis, USA, [5]MD Anderson Cancer Hospital, Houston, USA

Aim: Covalent (c) Bruton tyrosine kinase inhibitors (BTKi) have transformed management of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), but they are not curative, sharing pharmacologic liabilities like low oral bioavailability and short half-life that may lead to suboptimal BTK target coverage, especially in rapidly proliferating tumors with high BTK protein turnover (CLL/SLL), which can manifest as acquired cBTKi resistance . Pirtobrutinib, a highly selective, non-covalent (reversible) BTKi, inhibits wildtype and C481-mutant BTK with equal low nM potency and has favorable oral pharmacology enabling continuous BTK inhibition throughout the dosing interval regardless of intrinsic BTK turnover rate. In phase 1/2 BRUIN, pirtobrutinib demonstrated promising durable overall response rates (ORR) and was well tolerated irrespective of prior therapy (including cBTKi), number of prior lines of therapy, BTK C481 mutation status, or reason for prior cBTKi discontinuation. We compare efficacy and tolerability of pirtobrutinib versus ibrutinib in patients with CLL/SLL.

Method: BRUIN CLL-314 (NCT05254743) is a global, phase 3, open-label, randomized study comparing pirtobrutinib with ibrutinib. ~650 BTKi-naïve patients, (treatment-naïve [up to 30%]/previously treated with other non-BTKi agents) will be randomized 1:1 to once-daily 200mg pirtobrutinib/or 420mg ibrutinib (continuous monotherapy), stratified by del(17p) status (yes/no) and number of prior lines of therapy (0/1/≥2). Enrollment is ongoing.

Adults with CLL/SLL requiring therapy per iwCLL 2018 criteria are eligible. Key exclusion criteria: prior exposure to any BTKi, use of some concomitant therapies including anticoagulants like warfarin and other vitamin K antagonists, significant cardiovascular disease, active infections, and other clinically significant conditions. The primary objective is to establish non-inferiority of pirtobrutinib versus ibrutinib by comparing ORR assessed by an independent review committee. Superiority of pirtobrutinib in progression-free and event-free survival are key secondary outcomes. Trial efficacy and safety will be monitored by an independent data monitoring committee.

Zanubrutinib demonstrates superior progression-free survival (PFS) vs ibrutinib for treatment of relapsed/refractory chronic lymphocytic leukemia and small lymphocytic lymphoma (R/R CLL/SLL): final analysis of randomized phase 3 ALPINE study

Marlton P[1,2], Brown J[3], Eichhorst B[4,5], Hillmen P[6], Lamanna N[7], O'Brien S[8], Tam C[9,10], Qiu L[11], Kaźmierczak M[12], Jurczak W[13], Zhou K[14], Šimkovič M[15,16], Mayer J[17,18], Gillespie-Twardy A[19], Ferrajoli A[20], Ganly P[21], Weinkove R[22,23], Grosicki S[24], Mital A[25], Robak T[26], Osterborg A[27,28], Yimer H[29], Salmi T[30], Wang M[31], Fu L[31], Li J[31], Wu K[31], Cohen A[31], Shadman M[32,33]

[1]Department of Haematology, Princess Alexandra Hospital and University of Queensland, Brisbane, Australia, [2]University of Queensland School of Medicine, Brisbane, Australia, [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA, [4]Department I of Internal Medicine, University of Cologne, Cologne, Germany, [5]Center for Integrated Oncology Aachen, Bonn, Cologne, Düsseldorf, Germany, [6]St James's University Hospital, Leeds, UK, [7]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, USA, [8]Chao Family Comprehensive Cancer Center, University of California, Irvine, USA, [9]The Alfred Hospital, Melbourne, Australia, [10]Monash University, Melbourne, Australia, [11]Department of Lymphoma and Myeloma, Blood Diseases Hospital & Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China, [12]Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznan, Poland, [13]Maria Sklodowska-Curie National Research Institute of Oncology, Krakow, Poland, [14]First Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China, [15]4th Department of Internal Medicine - Hematology, University Hospital, Hradec Králové, Czech Republic, [16]Faculty of Medicine, Charles University, Prague, Czech Republic, [17]Department of Internal Medicine-Hematology and Oncology, University Hospital Brno, Brno, Czech Republic, [18]Faculty of Medicine, Masaryk University, Brno, Czech Republic, [19]Blue Ridge Cancer Care, Roanoke, USA, [20]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, USA, [21]Department of Haematology, Christchurch Hospital, Christchurch, New Zealand, [22]Te Rerenga Ora Blood and Cancer Centre, Te Whatu Ora Health New Zealand Capital Coast & Hutt Valley, Wellington, New Zealand, [23]Cancer Immunotherapy Programme, Malaghan Institute of Medical Research, Wellington, New Zealand, [24]Department of Hematology and Cancer Prevention, Health Sciences Faculty, Medical University of Silesia, Katowice, Poland, [25]Department of Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland, [26]Medical University of Lodz, Lodz, Poland, [27]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden, [28]Department of Hematology, Karolinska University Hospital, Stockholm, Sweden, [29]Texas Oncology-Tyler/US Oncology Research, Tyler, USA, [30]BeiGene International, GmbH, , Switzerland, [31]BeiGene (Beijing) Co., Ltd., Beijing, China; BeiGene USA, Inc., San Mateo, USA, [32]Fred Hutchinson Cancer Center, Seattle, USA, [33]Department of Medicine, University of Washington, Seattle, USA

Aim: Zanubrutinib, a more selective, next-generation Bruton tyrosine kinase inhibitor (BTKi) with improved BTK occupancy across disease-relevant tissues, was shown to be superior to the first-generation BTKi, ibrutinib, in the primary endpoint of overall response rate (ORR) by both independent review committee (IRC) and investigator in the predefined interim analysis of the phase 3 ALPINE trial (NCT03734016). Here, data from the predefined final analysis of the key secondary efficacy endpoint of PFS are reported.

Method: In ALPINE, patients with R/R CLL/SLL who received ≥1 prior therapy and had measurable disease were randomized 1:1 to receive zanubrutinib (n=327) or ibrutinib (n=325) until disease progression or unacceptable toxicity. As zanubrutinib was assessed as superior to ibrutinib in predefined interim analysis of the ORR primary endpoint, the key secondary efficacy endpoint, PFS, was assessed by hierarchical testing after 205 PFS events were reached. If zanubrutinib noninferiority was shown, superiority of zanubrutinib over ibrutinib could be tested and supported at a 2-sided *P* value of <.04996. Other endpoints included overall survival (OS) and safety.

Results: At data cutoff (August 8, 2022; median follow-up, 29.6 months), PFS per IRC was superior with zanubrutinib vs ibrutinib in the intention-to-treat population (hazard ratio, 0.65; *P*=.0024) (**Table**). Across major predefined subgroups, PFS by IRC and investigator consistently favored zanubrutinib over ibrutinib, including in patients with *del(17p)/TP53* mutation. The hazard ratio for OS with zanubrutinib vs ibrutinib was 0.76 (95% CI, 0.51-1.11). For multiple safety variables, rates were lower with zanubrutinib than with ibrutinib (**Table**).

Conclusion: ALPINE is the first study to show PFS superiority with zanubrutinib in a head-to-head comparison of BTKis in patients with R/R CLL/SLL. These data, with those from the predefined interim analysis, show that zanubrutinib had superior ORR and PFS and a favorable safety profile vs ibrutinib in patients with R/R CLL/SLL.

Table. PFS and Safety Results

	Zanubrutinib	Ibrutinib	Hazard ratio (95% CI)
PFS[a] in intention-to-treat population[b]			
Events, n (%)	88 (26.9)	120 (36.9)	0.65 (0.49-0.86); *P*=.0024[c]
Median (95% CI), months	Not reached	35.0 (33.2-44.3)	-
Rate at 24 months, %	79.5	67.3	-
PFS[a] in patients with *del17p/TP53* mutation[d]			
Events, n (%)	23 (30.7)	34 (45.3)	0.52 (0.30-0.88); *P*=.0134[e]
Rate at 24 months, %	77.6	55.7	-
Overall treatment discontinuation, %	26.3	41.2	-
Treatment discontinuation due to cardiac disorders, %	0.3	4.3	-
Grade 5 AEs due to cardiac disorders, %	0.0	1.9	-
Grade ≥3 AEs, %	67.3	70.4	-
Serious AEs, %	42.0	50.0	-
Atrial fibrillation/flutter, %	5.2	13.3	-
Dose interruption, %	50.0	56.8	-
Dose reduction, %	12.3	17.0	-
Death, %	14.7	18.5	-

AE, adverse event; PFS, progression-free survival. [a] By independent review committee; [b] Zanubrutinib, n=327; ibrutinib, n=325; [c] Two-sided *P* value; [d]Zanubrutinib, n=75; ibrutinib, n=75; [e] Nominal *P* value.

Zanubrutinib vs bendamustine + rituximab (BR) in patients with treatment-naive (TN) chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): extended follow-up of the SEQUOIA study

Opat S[1], Munir T[2], Shadman M[3], Robak T[4], Brown J[5], Kahl B[6], Ghia P[7], Giannopoulos K[8,9], Šimkovič M[10], Österborg A[11], Laurenti L[12], Walker P[13], Ciepluch H[14], Greil R[15,16,17], Hanna M[18], Tani M[19], Trněný M[20], Brander D[21], Flinn I[22], Grosicki S[23], Verner E[24,25], Tedeschi A[26], De Guibert S[27], Tumyan G[28], Laribi K[29], García J[30], Li J[31], Tian T[32], Ramakrishnan V[32], Liu Y[32], Szeto A[32], Paik J[32], Cohen A[32], Tam C[33], Jurczak W[34]

[1]Monash Health and Monash University, Clayton, Australia, [2]Haematology, Haematological Malignancy Diagnostic Service (HMDS), St. James's Institute of Oncology, Leeds, UK, [3]Fred Hutchinson Cancer Research Center, University of Washington, Seattle, USA, [4]Medical University of Lodz, Lodz, Poland, [5]Dana-Farber Cancer Institute, Boston, USA, [6]Washington University School of Medicine, St Louis, USA, [7]Strategic Research Program on Chronic Lymphocytic Leukemia, Division of Experimental Oncology, IRCCS Ospedale San Raffaele and Università Vita-Salute San Raffaele, Milan, Italy, [8]Experimental Hematooncology Department, Medical University of Lublin, Lublin, Poland, [9]Hematology Department, St John's Cancer Centre, Lublin, Poland, [10]4th Department of Internal Medicine – Haematology, Faculty of Medicine in Hradec Králové, University Hospital and Charles University in Prague, Hradec Králové, Czech Republic, [11]Department of Hematology, Karolinska University Hospital Solna, Stockholm, Sweden, [12]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, [13]Peninsula Health and Peninsula Private Hospital, Frankston, Australia, [14]Department of Hematology, Copernicus Regional Oncology Centre, Gdansk, Poland, [15]Third Medical Department with Hematology, Medical Oncology, Rheumatology and Infectiology, Paracelsus Medical University, Salzburg, Austria, [16]Salzburg Cancer Research Institute Center for Clinical Cancer and Immunology Trials, Salzburg, Austria, [17]Cancer Cluster Salzburg, Salzburg, Austria, [18]Department of Haematology, Waitemata District Health Board, Takapuna, New Zealand, [19]Hematology Unit, Santa Maria delle Croci Hospital, Ravenna, Italy, [20]First Department of Medicine, First Faculty of Medicine, Charles University, General Hospital, Prague, Czech Republic, [21]Hematologic Malignancies and Cellular Therapy, Duke University School of Medicine, Durham, USA, [22]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, USA, [23]Department of Hematology and Cancer Prevention, School of Public Health, Silesian Medical University, Katowice, Poland, [24]Concord Repatriation General Hospital, Concord, Australia, [25]University of Sydney, Sydney, Australia, [26]Department of Hematology, Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [27]Hôpital de Pontchaillou, Rennes, France, [28]Department of Chemotherapy of Hemoblastosis, Blokhin Russian Cancer Research Center, Moscow, Russia, [29]Hematology Department, Centre Hospitalier du Mans, Le Mans, France, [30]Laboratorio de Citometría de Flujo, Servicio de Hematología y Hemoterapia, Hospital Universitario de Getafe, Getafe, Spain, [31]Department of Hematology, Jiangsu Province Hospital, Nanjing, China, [32]BeiGene USA, San Mateo, USA, [33]Alfred Health and Monash University, Melbourne, Australia, [34]Maria Skłodowska-Curie National Research Institute of Oncology, Krakow, Poland

Aim: In SEQUOIA (NCT03336333), zanubrutinib displayed superior PFS vs BR in patients with TN CLL/SLL without del(17p); patients with del(17p) treated with zanubrutinib monotherapy showed similar outcomes. We report updated results from SEQUOIA (further 18 months of follow-up).

Method: Patients without del(17p) were randomized to receive zanubrutinib or BR; patients with del(17p) received zanubrutinib monotherapy. PFS, OS, ORR, and safety were evaluated. AEs were recorded until progression or start of next-line therapy.

Results: As of 31 October 2022, 479 patients without del(17p) were randomized to zanubrutinib (n=241) or BR (n=238). Median follow-up was 43.7 months (range, 0-60). Median PFS was not reached with zanubrutinib and 42.2 months with BR (HR, 0.30; 95% CI, 0.21-0.43). Estimated PFS rate at 42 months with zanubrutinib was 82.4%. PFS improved with zanubrutinib vs BR in patients with mutated *IGHV* (HR, 0.35; 95% CI, 0.19-0.64) and was sustained in patients with unmutated *IGHV* (HR, 0.23; 95% CI, 0.14-0.37). CR/CRi rates in patients without del(17p) were 17.4% (zanubrutinib) and 21.8% (BR). Median OS was not reached in either arm (HR, 0.87; 95% CI, 0.50-1.48); OS at 42 months was 89.4% (zanubrutinib) and 88.3% (BR). In 110 patients with del(17p), median follow-up was 47.9 months; 42-month PFS and OS rates were 79.4% and 89.5%, respectively, and the CR/CRi rate was 14.5%. Common causes of treatment discontinuation were AEs and progressive disease in those without (14.9% and 5.8%, respectively) and with del(17p) (13.5% each). Grade ≥3 AEs of interest included bleeding (5.8%, 1.8%), infection (23.8%, 22.0%), anemia (0.4%, 2.2%), thrombocytopenia (2.1%, 7.9%), and neutropenia (12.5%, 51.1%).

Conclusion: Extended follow-up SEQUOIA data showed that the efficacy and safety of zanubrutinib were maintained in patients without del(17p). Longer follow-up showed benefit in patients with mutated *IGHV.* Patients with del(17p) continued to demonstrate PFS benefit consistent with the randomized cohort.

Ibrutinib for treatment of relapsed-refractory chronic lymphocytic leukemia: a matching-adjusted indirect comparison of 3 randomized phase 3 trials

Seymour J[1], Ghia P[2], Munir T[3], Burger J[4], Rogers K[5], Huang H[6], Moreno C[7], Neumayr L[6], Abbazio C[6], Sharman J[8]

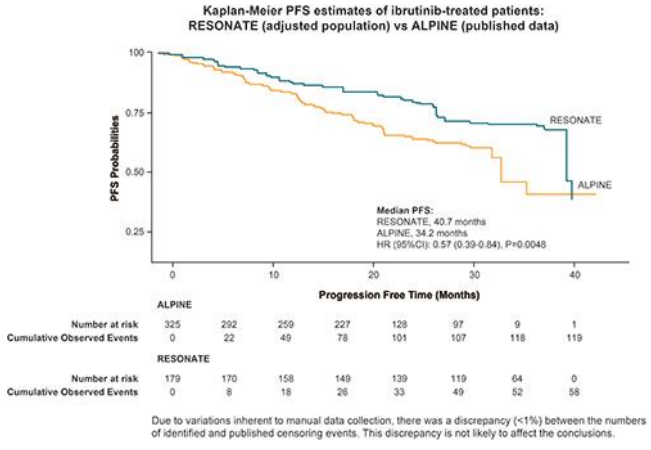
[1]Peter Maccallum Cancer Centre And The Royal Melbourne Hospital, Melbourne , Australia, [2]Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, Milan, Italy, [3]St James's University Hospital, Leeds, United Kingdom, [4]The University of Texas MD Anderson Cancer Center, Houston, USA, [5]The Ohio State University, Columbus, USA, [6]AbbVie, North Chicago, USA, [7]Hospital de la Santa Creu i Sant Pau, Josep Carreras Leukemia Research Institute, Autonomous University of Barcelona, Barcelona, Spain, [8]Willamette Valley Cancer Institute and Research Center, Eugene, USA

Aim: Ibrutinib is a standard therapy in first-line and relapsed-refractory (R/R) CLL. Our goal was to evaluate ibrutinib performance in R/R CLL by comparing its efficacy across the RESONATE, ALPINE, and ELEVATE-RR clinical trials.

Method: Individual patient data (IPD) of ibrutinib-treated patients from RESONATE (NCT01578707; n=195) were separately match-adjusted to ibrutinib-treated arms of ALPINE (NCT03734016; n=325) and ELEVATE-RR (NCT02477696; n=265) for key baseline characteristics: age ≥75 years, bulky disease, ≥3 or ≥4 prior treatments, β2-microglobulin >3.5 mg/L, and del(11q) or del(17p). After adjustment, the effective RESONATE sample size was n=95 (vs ALPINE) and n=69 (vs ELEVATE-RR). Adjusted ORR and PFS from RESONATE were compared with published outcomes from ALPINE and ELEVATE-RR; IPD for PFS were extracted from published Kaplan-Meier curves. Hazard ratios (HRs) were calculated using a weighted Cox model.

Results: Median follow-up was 36.0 vs 29.6 months (RESONATE adjusted vs ALPINE published) and 36.1 vs 40.9 months (RESONATE adjusted vs ELEVATE-RR published). Two-year PFS (95% CI) in RESONATE and ALPINE was 81% (74-90%) and 66% (60-71%), respectively; median PFS was 40.7 and 34.2 months (HR [95% CI] 0.57 [0.39-0.84] favoring RESONATE, *P*=0.0048); ORR (95% CI) was 90% (86-94%) and 74% (69-79%) (*P*<0.0001). Compared with ELEVATE-RR, RESONATE had greater 2-year PFS (79% [69-89%] vs 69% [64-75%], not statistically significant). Median PFS was 41.2 vs 44.1 months (HR [95%CI] 0.85 [0.55-1.31], *P*=0.46), and ORR was 89% (83-95%) and 80% (75-85%) (*P*=0.0381).

Conclusion: In phase 3 randomized trials in R/R CLL, ibrutinib was associated with robust PFS and ORR benefits. Ibrutinib outcomes were consistent between RESONATE and ELEVATE-RR; however, significant differences in ibrutinib performance were identified between RESONATE vs ALPINE. Despite the known limitations inherent to indirect comparisons, these results highlight the need to investigate elements of protocol design, center selection, or treatment delivery that may impact BTKi trial performance.



Zanubrutinib vs ibrutinib in relapsed/refractory chronic lymphocytic leukemia and small lymphocytic lymphoma (R/R CLL/SLL): impact on health-related quality of life (HRQOL)

Tam C[1], Eichhorst B[2,3], Lamanna N[4], O'Brien S[5], Qiu L[6], Yang K[7], Wu K[7,8], Salmi T[9], Barnes G[7], Brown J[10]
[1]Alfred Health and Monash University, Melbourne, Australia, [2]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany, [3]Center for Integrated Oncology, University Hospital Cologne, Cologne, Germany, [4]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, USA, [5]Chao Family Comprehensive Cancer Center, University of California, Irvine, USA, [6]Department of Lymphoma and Myeloma, Blood Diseases Hospital & Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China, [7]BeiGene USA, Inc., San Mateo, USA, [8]BeiGene (Beijing) Co., Ltd., Beijing, China, [9]BeiGene International GmbH, Basel, Switzerland, [10]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA

Aim: Assess HRQOL in patients treated with zanubrutinib vs ibrutinib.

Method: In the ALPINE study (NCT03734016), patients were randomized to zanubrutinib (n=327) or ibrutinib (n=325), and patient-reported outcome (PRO) endpoints (global health status [GHS], physical and role functions, fatigue, pain, diarrhea, and nausea/vomiting) were measured by EORTC QLQ-C30 and EQ-5D-5L at baseline, cycle 1, and every third 28-day cycle until end of treatment. Descriptive analyses using a mixed model for repeated measures of key PRO endpoints at cycle 7 (6 months) and cycle 13 (12 months) are presented.

Results: Patients had similar baseline characteristics and HRQOL at baseline. 15.4% of patients discontinued zanubrutinib due to adverse events vs 22.2% for ibrutinib. Adjusted PRO completion rates (the number of patients who completed the questionnaire divided by the number still on treatment) at cycles 7 and 13 were high with zanubrutinib (89.6% and 94.3%) and ibrutinib (87.7% and 92.3%). Zanubrutinib improved GHS scores vs ibrutinib at cycle 7 (least-squares mean change difference, 3.0; 95% CI, 0.23-5.77; nominal *P*=.0338) but not at cycle 13. Lower diarrhea scores and clinically meaningful improvements (≥5% mean change difference from baseline) in physical and role functioning, pain, and fatigue at cycles 7 and 13 were seen in the zanubrutinib arm (**Table**), but the difference between arms was not significant. Nausea/vomiting scores were maintained in both arms, with no measurable difference.

Conclusion: In ALPINE, patients with R/R CLL/SLL receiving zanubrutinib vs ibrutinib demonstrated improvement in GHS at cycle 7 (6 months). Improvement in other endpoints over time suggests that treatment with zanubrutinib positively affected HRQOL; however, given the generally good HRQOL at baseline in both arms, the differences between arms were small and not significant.

Table. Least-Squares Mean Change (95% CI) From Baseline Within Treatment Arms

	Cycle 7 (6 months)		Cycle 13 (12 months)	
	Zanubrutinib	**Ibrutinib**	**Zanubrutinib**	**Ibrutinib**
GHS	8.18 (6.25-10.12)	5.18 (3.20-7.17)	7.28 (5.41-9.15)	5.93 (3.97-7.89)
Physical functioning	6.55 (4.96-8.15)	4.73 (3.08-6.38)	5.46 (3.87-7.04)	4.31 (2.65-5.97)
Role functioning	6.95 (4.85-9.06)	6.32 (4.14-8.50)	6.81 (4.61-9.02)	5.01 (2.69-7.33)
Fatigue[a]	−12.54 (−14.47 to −10.60)	−10.63 (−12.63 to −8.62)	−11.13 (−13.19 to −9.08)	−10.78 (−12.93 to −8.63)
Nausea/vomiting[a]	−1.21 (−2.03 to −0.38)	−0.92 (−1.77 to −0.07)	−0.92 (−1.94 to 0.10)	−0.40 (−1.47 to 0.66)
Pain[a]	−5.06 (−7.21 to −2.91)	−3.63 (−5.85 to −1.42)	−5.18 (−7.38 to −2.97)	−2.75 (−5.06 to −0.44)
Diarrhea[a]	−2.11 (−3.80 to −0.42)	−0.52 (−2.27 to 1.22)	−3.23 (−4.79 to −1.66)	−1.38 (−3.03 to 0.27)

Data cutoff: August 8, 2022; GHS, global health status; [a] Negative values indicate improvement.

Characterization of the safety/tolerability profile of zanubrutinib and comparison with the profile of ibrutinib in patients with B-cell malignancies: post hoc analysis of a large clinical trial safety database

Tam C[1], Brown J[2], Eichorst B[3,4], Ghia P[5], Jurczak W[6], Kahl B[7], Lamanna N[8], Robak T[9], Shadman M[10,11], Qiu L[12], Cohen A[13], Zhang M[13], Salmi T[14], Paik J[13], Wang L[13], Zhang J[13], Ma H[13], Tedeschi A[15]

[1]Alfred Health and Monash University, Melbourne, Australia, [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA, [3]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany Center for Integrated Oncology, University Hospital Cologne, Cologne, Germany, [4]Center for Integrated Oncology, University Hospital Cologne, Cologne, Germany, [5]Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, Milano, Italy, [6]Maria Sklodowska-Curie National Research Institute of Oncology, Krakow, Poland, [7]Siteman Cancer Center, Washington University School of Medicine, St. Louis, USA, [8]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, USA, [9]Medical University of Lodz, Lodz, Poland, [10]Fred Hutchinson Cancer Research Center, Seattle, USA, [11]Department of Medicine, University of Washington, Seattle, USA, [12]Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Tianjin, China, [13]BeiGene USA, Inc, San Mateo, USA, [14]BeiGene International GmbH, Basel, Switzerland, [15]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

Aim: To characterize the overall safety and tolerability of zanubrutinib, a potent and selective next-generation Bruton tyrosine kinase inhibitor (BTKi), in patients with B-cell malignancies and compare its profile with that of the first-generation BTKi, ibrutinib.

Method: In the post hoc analyses, safety data were pooled from 10 zanubrutinib monotherapy clinical trials in patients with CLL/SLL, MCL, MZL, WM, FL, and other B-cell malignancies (N=1550), including 2 (ASPEN, ALPINE) that compared zanubrutinib head-to-head with ibrutinib. Incidence rates and exposure-adjusted incidence rates (EAIRs) of treatment-emergent adverse events (TEAEs; summarized in MedDRA preferred terms) and adverse events of special interest (AESIs; defined in grouped terms) were assessed.

Results: Median zanubrutinib exposure was 34.4 months. The most common nonhematologic any-grade TEAEs with zanubrutinib were upper respiratory tract infection (29.7%), diarrhea (21.1%), contusion (19.5%), cough (18.1%), and rash (16.6%). Grade ≥3 TEAEs in ≥5% of patients included pneumonia (8.4%) and hypertension (8.1%). The only serious TEAE in ≥5% of patients was pneumonia (8.2%). In ASPEN/ALPINE, patients treated with zanubrutinib had lower rates of discontinuation (14.1% vs 22.0%), dose reduction (13.9% vs 19.2%), and death (8.7% vs 10.2%) due to TEAEs than those treated with ibrutinib. EAIRs of AESIs were numerically lower with zanubrutinib vs ibrutinib, except for neutropenia (**Table**). With longer follow-up, the prevalence of AESIs with zanubrutinib generally remained constant or decreased.

Conclusion: These pooled safety analyses in patients with B-cell malignancies showed that zanubrutinib is well tolerated, with generally mild to moderate TEAEs and low discontinuation rates due to TEAEs. AESI prevalence generally decreased over time, with no new safety signals emerging. Long-term tolerability and low discontinuation rates with BTKis are important because continuous treatment is required for better outcomes. These analyses support zanubrutinib as an appropriate long-term treatment option for patients with B-cell malignancies.

Table. Exposure-Adjusted Incidence Rates for Adverse Events of Special Interest

	Zanubrutinib (N=1550)	ASPEN/ALPINE[a]	
		Zanubrutinib (n=425)	Ibrutinib (N=422)
Median exposure, mo	34.4	32.6	25.7
Exposure-adjusted incidence rate, person/100 person-months			
Infections	6.01	5.40	6.64
Opportunistic infections	0.07	0.07	0.13
Hemorrhage	3.00	2.49	3.00
Major hemorrhage	0.17	0.17	0.24
Neutropenia	1.21	1.32	1.05
Thrombocytopenia	0.59	0.49	0.65
Hypertension	0.57	0.82	1.08
Anemia	0.51	0.57	0.75
Second primary malignancies	0.52	0.47	0.58
Skin cancers	0.30	0.27	0.38
Atrial fibrillation/flutter	0.15	0.20	0.64

[a] Head-to-head trials of zanubrutinib vs ibrutinib.

Rapid and deep responses with asciminib in patients with chronic myeloid leukemia in chronic phase (CML-CP) after ≥2 prior tyrosine kinase inhibitors in the phase 3 ASCEMBL study

Khanna A[1], Hughes T[2], Réa D[3], Boquimpani C[4], Minami Y[5], Mauro M[6], Cortes J[7], Apperley J[8], Garcia-Gutiérrez V[9], Kapoor S[10], Dawson M[11], Dhamal V[12], Hochhaus A[13]

[1]Novartis Pharmaceuticals Australia Pty Ltd, Sydney, Australia, [2]South Australian Health and Medical Research Institute and University of Adelaide, Adelaide, Australia , [3]Hôpital Saint-Louis, Paris, France , [4]HEMORIO, State Institute of Hematology Arthur de Siquiera Cavalcanti, Rio de Janeiro, Brazil , [5]National Cancer Center Hospital East, Kashiwa, Japan , [6]Memorial Sloan Kettering Cancer Center, New York, USA , [7]Georgia Cancer Center, Augusta, USA , [8]Centre for Haematology Imperial College London, London, UK , [9]Servicio de Hematología, Hospital Universitario Ramón y Cajal (IRYCIS), Madrid, Spain , [10]Novartis Pharmaceuticals Corporation, East Hanover, USA , [11]Novartis Pharma AG, Basel, Switzerland , [12]Novartis Healthcare Private Limited,, Hyderabad, India , [13]Universitätsklinikum Jena, Jena, Germany

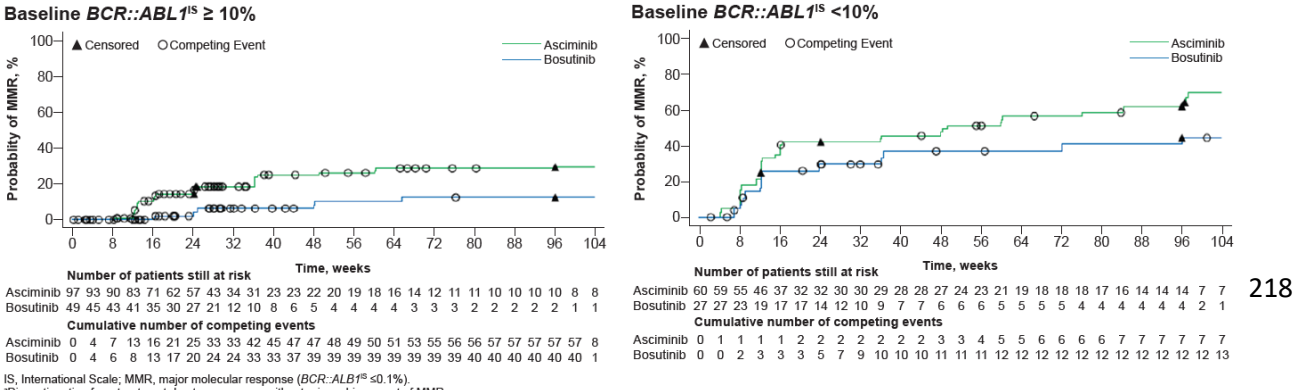
Aim: In ASCEMBL (NCT03106779), after ≥2y follow-up, superiority of asciminib versus bosutinib was demonstrated in CML-CP. We characterized the efficacy of asciminib versus bosutinib through exploratory analyses.

Method: Patients with intolerance/resistance to ≥2 TKIs were randomized 2:1 to asciminib 40mg BID or bosutinib 500mg QD. Patients with *BCR::ABL1*^{IS} >10% at week (wk) 24 discontinued therapy due to lack of efficacy. Analyses included: molecular response per baseline *BCR::ABL1*^{IS}; cumulative incidence of major molecular response (MMR)/*BCR::ABL1*^{IS} ≤1% in nonresponders by wk24; MMR at wk96 by number of prior 2G TKIs; cumulative incidence of MMR by baseline *BCR::ABL1*^{IS}, reason for prior TKI discontinuation, and line of therapy (cutoff: October 6, 2021).

Results: Among asciminib-treated patients with baseline *BCR::ABL1*^{IS} ≤10%, 18 (30.0%), 24 (40.0%), and 36 (60.0%) reached MMR at wk12, 24, and 96; 38 (63.3%), 45 (75.0%), and 47 (78.3%) reached *BCR::ABL1*^{IS} ≤1%, respectively. Of 97 with baseline *BCR::ABL1*^{IS} >10%, 10 (10.3%), 16 (16.5%), and 23 (23.7%) reached MMR; 28 (28.9%), 32 (33.0%), and 29 (29.9%) reached *BCR::ABL1*^{IS} ≤1%, respectively. Twenty-three (14.6%) had *BCR::ABL1*^{IS} >10% at wk24 and discontinued treatment. Of 56 asciminib-treated patients without MMR by wk24, MMR (95% CI) probability was 17.9% (9.1%–29.0%) and 37.9% (25.1%–50.6%) by 1 and 2years, respectively. At wk96, MMR was higher with asciminib versus bosutinib in patients resistant to 1 (34.3% vs 7.7%) or ≥2 (35.0% vs 16.7%) prior 2G TKIs. Cumulative incidence of MMR was consistently higher with asciminib than bosutinib regardless of baseline *BCR::ABL1*^{IS} (Figure). By wk96, cumulative incidence of MMR was higher with asciminib than bosutinib in patients who discontinued prior TKI due to lack of efficacy (33.2% vs 9.4%), even in the third- (45.9% vs 33.3%) and fourth-line (40.9% vs 24.8%) setting.

Conclusion: Responses were fast with asciminib and continued to deepen over time; MMR might be achieved beyond wk24.

Figure: Cumulative incidence of MMR adjusting for competing risks[a] by baseline *BCR::ABL1*^{IS} levels



IS, International Scale; MMR, major molecular response (*BCR::ABL1*^{IS} ≤0.1%).
[a]Discontinuation from treatment due to any reason without prior achievement of MMR

Chronic myeloid leukemia survey on unmet needs (CML SUN): balancing tolerability and efficacy goals of patients and physicians through shared treatment decision-making

Khanna A[1], Lang F[2], Clements J[3], Ruiz C[3], Réa D[4], Machado L[5], Takahashi N[6], Moon S[7], Grigg A[8], Borowczak C[9], Schuld P[10], Frank P[10], Constantinescu C[11], Boquimpani C[12,13], Cortes J[14]
[1]Novartis Pharmaceuticals Australia Pty Ltd, Sydney, Australia, [2]Goethe University Hospital, Frankfurt am Main, Germany, [3]CML Buster Foundation, Costa Mesa, USA, [4]Hôpital Saint-Louis, Paris, France, [5]Canadian CML Network, Toronto, Canada, [6]Akita University Graduate School of Medicine, Akita, Japan, [7]Korea Leukemia Patients Organization, Seoul, South Korea, [8]Austin Hospital, Melbourne, Australia, [9]LeukaNET, Hohenbrunn, Germany, [10]Novartis Pharma AG, Basel, Switzerland, [11]IPSOS, Basel, Switzerland, [12]State Institute of Hematology Arthur de Siqueira Cavalcanti, Rio de Janeiro, Brazil, [13]Oncoclínica Centro de Tratamento Oncológico, Rio de Janeiro, Brazil, [14]Georgia Cancer Center, Augusta University, Augusta, USA

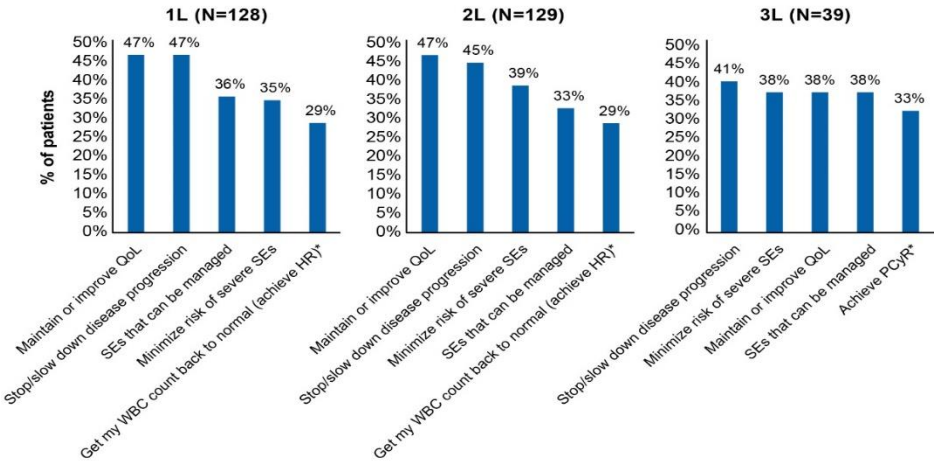
Aim: Data on patient experiences/concerns regarding outcomes and role in treatment decision-making in CML are lacking. CML SUN aimed to understand the unmet needs/concerns around CML from the perspective of patients and haematologists.

Method: CML SUN was conducted among patients with CML in chronic phase (CML-CP; aged ≥18 years; receiving a second or later-line TKI) and haematologists (treated ≥10 patients with CML-CP over the last year). Qualitative interviews were used to inform the quantitative surveys questions (specific for patients and doctors) reported here.

Results: This interim analysis reports responses from 130 patients and 150 doctors from 9 countries. For patients with CML, maintaining/improving QoL was the top goal in the first- and second-line settings; slowing disease progression was the priority in the third-line setting (Figure). Doctors emphasised efficacy across treatment lines with less focus on side effects (SEs). Only 49% of patients recalled receiving SE information and 34% of doctors reported not providing any. Many doctors (49%) presented patients with one treatment option at diagnosis; reasons included perceptions about patients' decision-making capacity. Across treatment lines, ~50% of doctors reported making treatment decisions with minimal/no patient input; one-third of patients reported involvement in their treatment decisions. Most patients were satisfied with treatment efficacy, but not with the SEs (41%). When switching treatment, patients' top goal was to achieve/maintain QoL, while doctors focused on response/long-term survival. During first treatment, one-third of patients report SEs only when asked. Of 35 patients who informed their doctor of SEs and switched treatment, 43% felt empathy from the doctor; 23% reported that their doctor considered the SEs non-serious and expected treatment continuation. Of 27 patients who reported current treatment noncompliance, SEs were the top reason.

Conclusion: Patients with CML may have different goals than haematologists, indicating a need for improved communication/shared decision-making, especially to manage SEs.

Figure. Top 5 Treatment goals for patients by line of therapy



In the 1L, 2L and 3L settings, 2, 1, and 1 patients who answered 'I don't remember' were excluded, respectively.
*Goals related to efficacy. 1L, first line; 2L, second line; 3L, third line; QoL, quality of life; SE, side effect; PCyR, partial cytogenetic response; WBC, white blood cells.

Matching-adjusted indirect comparison of asciminib versus other tyrosine kinase inhibitors in third-or-later line chronic-phase chronic myeloid leukemia (CML-CP)

Khanna A[1], Atallah E[2], Mauro M[3], Hochhaus A[4], Boquimpani C[5,10], Minami Y[6], Réa D[7], Yocolly A[8], Samjoo I[9], Kangappaden T[9]

[1]Novartis Pharmaceuticals Australia Pty Ltd, Sydney, Australia, [2]Medical College of Wisconsin, Milwaukee , USA, [3]Memorial Sloan-Kettering Cancer Center, New York, USA, [4]Universitätsklinikum Jena, Jena, Germany, [5]HEMORIO, State Institute of Hematology Arthur de Siquiera Cavalcanti, , Rio de Janeiro, Brazil, [6]National Cancer Center Hospital East, , Kashiwa, Japan, [7]Adult Hematology Hôpital Saint-Louis and FiLMC, Paris, France, [8]Novartis Services Inc., , East Hanover, USA, [9]EVERSANA, Burlington, Canada, [10]Oncoclínica Centro de Tratamento Oncológico, , Rio de Janeiro, Brazil

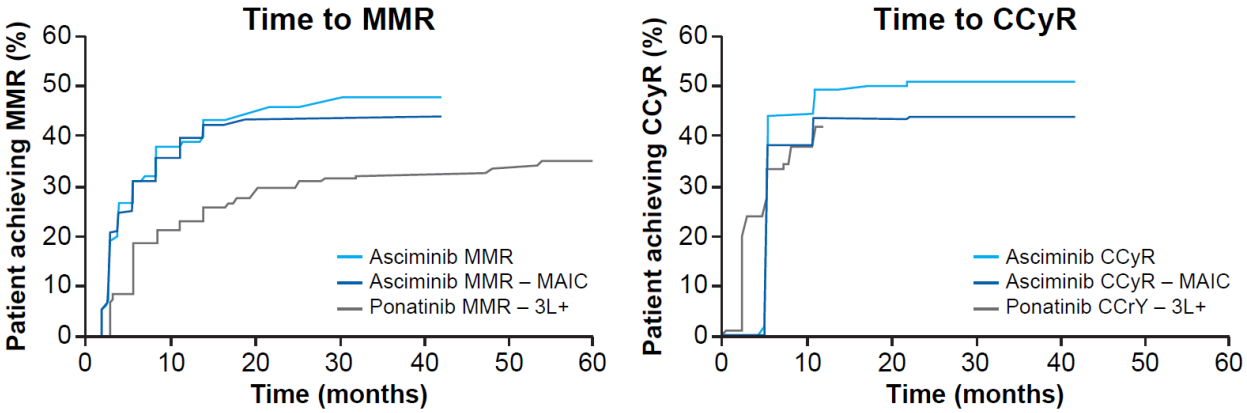
Aim: There is paucity of head-to-head TKI trials in CML-CP. Matching-adjusted indirect comparisons (MAICs) were conducted to compare asciminib with TKIs in third- or later-line (≥3L) in CML-CP.

Method: Unanchored MAICs were conducted to adjust individual patient-level data for asciminib (ASCEMBL [NCT03106779]; follow-up: ≥96 weeks) to published aggregate data for comparator TKIs (ponatinib [PACE: ≥3L cohort], nilotinib [Giles 2010; Ibrahim 2010], and dasatinib [Tan 2019; Rossi 2013; Ibrahim 2010]). Where feasible, major molecular response (MMR), complete cytogenic response (CCyR), and times to MMR and CCyR for asciminib and TKIs were assessed. Additional sensitivity analyses were conducted (e.g., removing ponatinib pretreated patients).

Results: Asciminib was associated with statistically significant superiority in MMR by 6 (relative risk [RR]: 1.55; 95% CI: 1.02, 2.36) and 12 months (RR: 1.48; 95% CI: 1.03, 2.14) versus ponatinib. There was no difference for CCyR, by 6 (RR: 1.11; 95% CI: 0.81, 1.52) or 12 months (RR: 0.97; 95% CI: 0.73, 1.28) versus ponatinib. Removing ponatinib pretreated patients showed higher responses in MMR by 6 (RR: 1.68; 95% CI: 1.10, 2.55]) and 12 months (RR: 1.72; 95% CI: 1.20, 2.45) and numerical improvements in CCyR by 6 (RR: 1.15; 95% CI: 0.84, 1.59) and 12 months (RR: 1.03; 95% CI: 0.78, 1.36) for asciminib versus ponatinib; furthermore, sensitivity analyses showed favorable results for time to MMR and CCyR by 6 and 12 months for post-adjustment asciminib versus ponatinib (Figure). Asciminib was associated with statistically significant improvements in CCyR by 6 (RR: 3.57; 95% CI: 1.42, 8.98) and 12 months (RR: 2.03; 95% CI: 1.12, 3.67) versus pooled nilotinib/dasatinib. There were no differences for asciminib in MMR by 6 months versus dasatinib (RR 1.52; 95% CI: 0.66, 3.53).

Conclusion: Results of the MAICs showed comparable outcomes for asciminib in MMR, and CCyR versus other TKIs in CML-CP.

Figure: Time to MMR (left) and CCyR (right) for asciminib vs ponatinib (sensitivity analysis*; N=30; ESS=38)



*Sensitivity analysis #1: Excluded all the 67 patients who either had CCyR at baseline or for whom CCyR information was not available at baseline and those who were pretreated with ponatinib (19+35+13).3L+, third or later line; CCyR, complete cytogenic response; ESS, effective sample size; MAIC, matching-adjusted indirect comparison; MMR, major molecular response.

Investigating lipid droplet metabolism and treatment resistance in chronic myeloid leukaemia cell lines

Tolland M[1,2], Collis E[1,2], Mah C[1,2], Ross D[1,2,3,4,5,6], Hughes T[1,2,3,4], Pagani I[1,2,3]
[1]Faculty of Health and Medical Sciences, University Of Adelaide, Adelaide, Australia, [2]South Australian Health and Medical Research Institute, Adelaide, Australia, [3]Australasian Leukaemia and Lymphoma Group, Melbourne , Australia , [4]Department of Haematology and Bone Marrow Transplantation, Royal Adelaide Hospital , Adelaide, Australia, [5]Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia , [6]Department of Haematology and Genetic Pathology, Flinders University and Medical Centre , Adelaide, Australia

Aim: While Tyrosine Kinase Inhibitors (TKIs) have improved Chronic Myeloid Leukaemia (CML) prognosis, most patients require lifelong treatment, and some display therapy resistance. This is due to persistent leukaemic cells not eradicated by TKI therapy. TKI-resistant leukaemic cells appear to undergo metabolic rewiring and we hypothesised that changes in lipid droplet (LD) metabolism may promote TKI resistance in CML.

Method: TKI-sensitive and resistant K562 cells were stained with neutral lipid dye, BODIPY 493/503 for flow cytometry analysis of LDs. Expression of LD regulatory gene, *ABHD5*, was measured in imatinib sensitive and 2μM imatinib resistant K562 cells by RT-qPCR. Since ABHD5 also promotes autophagy induction, autophagic flux was measured through western blot analysis of autophagy marker LC3BII. Cell viability following hydrogen peroxide (H_2O_2) induced oxidative damage was measured by AnnexinV/7-AAD staining for flow cytometry. Statistical significance was determined using t-tests and ANOVA testing.

Results: Interestingly, both imatinib and dasatinib resistant K562 cells displayed higher basal LD levels compared to their TKI-sensitive counterparts (p=0.0043 and p=0.034, respectively). Treatment with 0.5μM imatinib and 10nM dasatinib reduced LDs in both imatinib (Figure 1) and dasatinib sensitive cells (p=0.0008 and p=0.01, respectively) but not in the resistant cells. Moreover, imatinib treatment of sensitive cells, but not resistant cells, showed increased expression of *ABHD5* (1.7-fold, p<0.0001) which is involved in LD degradation and autophagy induction. This finding was consistent with increased autophagic flux following imatinib treatment in imatinib-sensitive cells (0.23-fold, p=0.025), with no change observed in imatinib resistant cells. Following combination H_2O_2/imatinib treatment, sensitive cells displayed 1.3-fold lower LD levels (p<0.0001) and increased lethality (p=0.0015) compared to the imatinib resistant cells (Figure 2).

Conclusion: Our study sheds light on a potential mechanism of resistance, not previously described in CML, indicating dysregulation in ABHD5-mediated LD degradation, which requires further investigation as a new therapeutic target in CML.

Figure 1: Lipid droplet levels following 48hr imatinib treatment

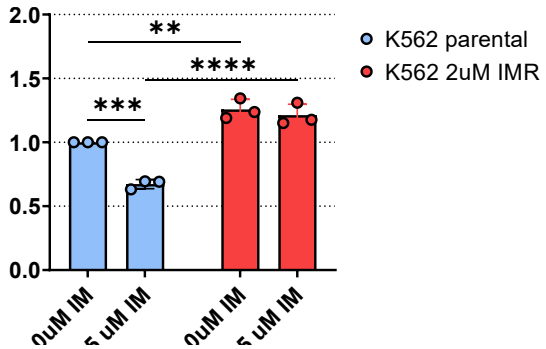
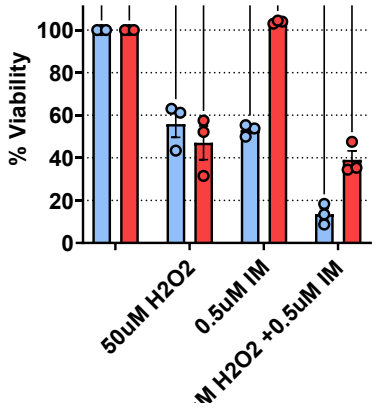


Figure 2: Cell viability following 72hr H_2O_2/imatinib combination treatment



Comparable b2a2/e13a2 and b3a2/e14a2 reporting in BCR-ABL assay when calibrated by WHO IS and by IVT-RNA copy number

Wei H[1], Tran T[1], Shridhar K[1], Wang N[1], Day G[1]

[1]*Cepheid, Sunnyvale, United States*

Aim: To establish a %CN e13a2/ABL and e14a2/ABL reporting method with known CN of IVT-RNAs and compare to WHO *IS* for e13a2/e14a2 breakpoint specific % reporting.

Method: Three IVT-RNAs (e13a2-ABL-BCR, e14a2-ABL-BCR and ABL-BCR) were used to generate standard curves for %CN reporting (Figure1). Four levels of IVT-RNA panels with same CN of e13a2 and e14a2 were tested for breakpoint specific % reporting comparison. K562(e14a2), BV173 (e13a2) cell lysates and CML clinical samples carrying e13a2 or e14a2 transcripts were tested to evaluate the %CN reporting compared to WHO *IS*.

Results: Good linearity demonstrated in Ct vs CN input for e13a2, e14a2 and ABL IVT-RNA (Figure2) with comparable efficiency (E) between e13a2 (E=0.992) and e14a2 (E=0.986). %e13a2 reporting was ~1.50-fold (by WHO *IS*) and ~1.46-fold (by %CN) higher than %e14a2, by testing IVT-RNA panel (Table1). Minor differences in %reporting observed between % CN and WHO *IS* for e13a2 (84.5%~110.8%) vs e14a2 (82.5%~89.5%) from cell lysates (Table2) and e13a2 (92.6%~105.5%) vs e14a2 (88.1%~92.2%) from clinical samples (Table3).

Conclusion: Both WHO *IS* and %CN showed very minor differences between e13a2 and e14a2 for % reporting. %CN method demonstrated comparable %reporting to WHO *IS.*

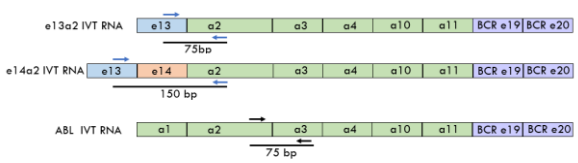


Figure 1: Schematic of e13a2-ABL-BCR, e14a2-ABL-BCR and ABL-BCR IVT-RNA with positions of primers

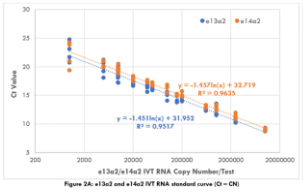


Figure 2A: e13a2 and e14a2 IVT RNA standard curve (Ct – CN)

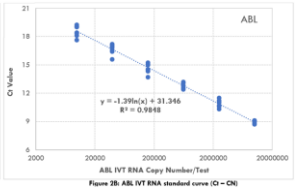


Figure 2B: ABL IVT RNA standard curve (Ct – CN)

IVT RNA Panel	e13a2/ABL % WHO *IS*	e13a2/ABL % CN	e14a2/ABL % WHO *IS*	e14a2/ABL % CN	% WHO *IS* e13a2/ e14a2	% CN e13a2/ e14a2
1	6.64	5.56	5.76	4.69	1.15	1.19
2	1.17	1.08	0.718	0.698	1.63	1.55
3	0.039	0.045	0.021	0.025	1.86	1.80
4	0.011	0.013	0.008	0.01	1.38	1.30

Table 1: e13a2 and e14a2 IVT RNA panel test

Cell lysates	BCR-ABL CN by std	ABL CN by std	% by WHO *IS*	% by CN	% WHO *IS*/% CN
BV-173 e13a2 level1	3608	583331	0.7368	0.6652	1.108
BV-173 e13a2 level2	346	662008	0.0556	0.0584	0.952
BV-173 e13a2 level3	43	657409	0.0057	0.0067	0.845
K562-e14a2 level1	6837	382436	1.4033	1.7006	0.825
K562-e14a2 level2	635	531496	0.1004	0.1168	0.860
K562-e14a2 level3	61	706370	0.0082	0.0092	0.895

Table 2: %CN and %WHO *IS* from unknown %e13a2 and %e14a2 cell lysates

CML Clinical Sample	e13a2/ e14a2	BCR-ABL CN by std	ABL CN by std	% By WHO *IS*	% By CN	% WHO IS/% CN
1	e13a2	464	354994	0.128	0.131	0.980
2	e13a2	271	546621	0.047	0.050	0.947
3	e13a2	7254	1296035	0.590	0.560	1.054
4	e13a2	14186	783262	1.910	1.811	1.055
5	e13a2	742	381474	0.180	0.194	0.926
6	e13a2	3033	1857111	0.170	0.163	1.041
7	e14a2	561	1392711	0.035	0.040	0.881
8	e14a2	340	1327815	0.022	0.025	0.888
9	e14a2	247	1296035	0.017	0.019	0.892
10	e14a2	66	1051983	0.0057	0.0063	0.922

Table 3: %WHO *IS* and %CN from e13a2 and e14a2 CML clinical samples

The incidence and complexity of variant Philadelphia chromosomes in chronic myeloid leukemia: The VCCS experience

Wong Y[1], Dun K[1], Ninkovic S[1], Mercer B[1]
[1]*Victorian Cancer Cytogenetics Service, Newport, Australia*

Aim: Approximately 5-10% of CML patients have variant form Ph chromosome, characterized by additional chromosome regions beyond 9 and 22. Previous studies have reported that the presence of variant Ph does not bestow a prognostic disadvantage when compared to the classic Ph.

The aim of this study is to report the incidence of variant Ph chromosome in newly diagnosed CML patients over a 16-year period in Victoria and compare their outcomes.

Method: We analysed our database from January 2007 to February 2023, identifying 1331 new cases of CML with 69 (5.2%) harbouring a variant Ph chromosome. Variants were classified based on complexity as either one-step (involving simultaneous breakage of multiple chromosomes resulting in three-way or more complicated reciprocal translocations) or two-step (classic Ph rearrangement followed by an additional translocation involving 22q11.1 or 9q34 locus). Clinical data are currently being collated. Statistical analysis will involve comparing dichotomous variables using the chi-squared test or Fisher's exact test.

Results: Among 69 patients with variant Ph chromosomes (58% male, n=777; 42% female, n=554), 45 (65%) exhibited one-step translocations, with 3 (4%) having a simple variant (translocation involving chromosome 22 and a chromosome other than 9), 36 (52%) displaying reciprocal three-way translocations, and 6 (9%) demonstrating reciprocal four or more-way translocations. The remaining 24 (35%) exhibited variants with a two-step mechanism. The most frequent chromosome partners involved in the variant translocations were chromosome 11, 17, and 19, with 8 cases observed for each. Among these, the 11q13 breakpoint was the most common, occurring in 5 cases. More clinical data will be presented upon collation.

Conclusion: The incidence of variant Ph chromosomes in our study (5.2%) aligns with published reports. However, a significant proportion of our variant Ph chromosomes were generated through a two-step mechanism. The recurring 11q13 breakpoint, also observed by others, is associated with known oncogenes or secondary breakpoints in other cancers.

Factors influencing lymphocyte collection efficiency for the manufacture of CART cells in adults with B cell malignancies , a single center experience

Belbachir S[1], Purtill D[1], Chiappini P[1], Scott C[1], Barraclough A[1]
[1]*Fiona Stanley Hospital, Perth, Australia*

Aim: During leukapheresis, accurate estimation of the proportion of T cells that are removed from peripheral blood (collection efficiency, CE) is essential to minimise apheresis time, reduce lab storage requirements and prevent excess product collection. Based on historical precedent from autologous HPC donors, many centres estimate a CE of 40% for autologous T cell donors, although data are lacking. We aimed to identify patient characteristics and collection parameters that might influence leukapheresis CE in order to maximise efficiency and minimize waste during CAR T manufacture.

Methods: We reviewed data from 33 consecutive patients who underwent leukapheresis for CAR-T cell treatment at our centre from March 2020 to October 2022. Apheresis was performed on the Spectra Optia, using the CMNC collection protocol.

Results: 33 apheresis episodes were recorded for patients with acute lymphocytic leukemia (n=1), NHL (n=27) and myeloma (n=5).
The median age and weight of patients was 62 years (18 -76) and 75 kg (45-136) respectively, with a male to female ratio of 1.38. The median prior lines of therapy was 2 (1-7) with 33% of pts receiving a prior autologous HCT and 3% allogeneic HCT.

The median WBC was 6×10^9/L (2–24), median haematocrit was 0.34 (0.25-0.45) and median absolute lymphocyte count (ALC) was 0.8 (0.21–2.4). Median CD3 value was 560×10^9/L and median percentage of ALC was 76%.

Collection was successful in all patients. The median CD3 lymphocyte count in the apheresis product was 4.3×10^9 and viability was 99%. The median CE was 71% (31-96) and 97% patients had a CE of >40%. Median apheresis run time was 210 minutes. Total blood and apheresis product volumes were 9750 and 278ml respectively. In the apheresis product, the median CD3 value was 15415×10^6 and the median CD3 value per kg was 58×10^6 /kg.

In multivariate analysis, baseline characteristics did not significantly impact the collection efficiency. ALC and CD3 pre-apheresis were associated with the CD3 yield after apheresis ($p < 0.001$). No major adverse events were recorded.

Conclusion: High efficiency leukapheresis is safe and feasible in autologous T-cell donors. Our centre now uses a CE estimate of 60% for collection of CAR T starting material.

In the absence of symptoms or cerebrospinal fluid involvement, MRI staging of the central nervous system (CNS) in patients with systemic diffuse large B-cell lymphoma rarely detects disease

Bennett R[1], Juneja M[1], Anderson M[1], Minson A[1], Wolf M[1], Herbert K[1], Blombery P[1], Mason K[1], Lieschke G[1], Roberts A[1], Seymour J[1], Dickinson M[1]
[1]*Royal Melbourne Hospital & Peter Maccallum Cancer Centre, Melbourne, Australia*

Background: While cerebrospinal fluid (CSF) analysis and cerebral magnetic resonance imaging (MRI) are used to screen high-risk DLBCL patients for synchronous CNS involvement (sCNS) at diagnosis, the utility of MRI is uncertain. We sought to describe institutional outcomes of CNS staging for high-risk DLBCL and the value of MRI adjunctive to CSF analysis.

Methods: We retrospectively analysed consecutive newly diagnosed patients 1/2011–11/2022 with DLBCL/high-grade B-cell lymphoma from institutional databases. Patients with IPI ≥3; ≥2 extra-nodal sites, or ≥1 extra-nodal site *and* elevated LDH; renal, adrenal, breast or testicular involvement; and/or double/triple-hit by FISH as defined by the WHO 2016 classification, with available CNS staging information, were included. Fisher exact tests were performed.

Results: 540 patients were identified; 167 met inclusion criteria. 139 (83%) underwent CSF analysis (n=133, 79.6%), MRI brain (n=74, 44.3%), or both (n=68, 40.7%). CNS involvement seen in 6/139; 4/6 positive MRI and CSF findings, 1/5 had positive MRI but negative CSF, 1/6 positive CSF (cytology) but no MRI performed. Proportions of positive MRI (33.3% vs. 0%, p=0.0002) and CSF (23.1% vs. 1.0%, p=0.0028) analyses were higher for symptomatic patients (n=16) than asymptomatic patients (n=150). Of asymptomatic patients with negative CSF analyses (n=117), 55 (47%) had MRI - none identified CNS involvement. Excluding sCNS, eight patients (5.0%) experienced CNS relapse after median 9.6 (range, 6.2-43.6) months, including six relapses in asymptomatic patients with negative CSF (5.1%) of whom had three baseline MRI (all negative). High-dose intravenous and/or intrathecal methotrexate CNS prophylaxis had been administered to 7/8 patients.

Summary/Conclusion: Presence of neurological symptoms is not specific for synchronous CNS involvement, but justifies CSF analysis and MRI staging. For asymptomatic patients, the utility of adjunctive cerebral MRI is very limited when CSF analysis is negative. More sensitive staging techniques, such as analyses of CSF cfDNA, are desirable.

VEXAS Syndrome associated with lymphoplasmacytic lymphoma

Biggart R[1], Sternberg A[1]
[1]*Great Western Hospital, Swindon, United Kingdom*

Abstract: A recent genotype-driven classification of haematological and rheumatological disease has been termed VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome. Acquired x-linked UBA1 variants are found, resulting in changes to ubiquitylation and regulation of cellular stress response. This adult onset autoinflammatory condition has been seen in association with myelodysplasia and plasma cell dyscrasias. This case report from the United Kingdom (UK) describes VEXAS syndrome for the first time in association with lymphoplasmacytic lymphoma (LPL).

A 58-year-old man underwent initial investigations and treatment for LPL with rituximab, cyclophosphamide and prednisolone. His anaemia remained out of proportion to his LPL infiltrate and he underwent serial bone marrow aspirations demonstrating significant trilineage dysplasia but no clonal abnormality by cytogenetics or myeloid next generation sequencing (NGS) panels. After developing arthralgia and relapsing polychrondritis he was investigated for a systemic autoinflammatory disorder and found to have a variant at p(Met41) of UBA1.

UBA1 testing has only recently become available on UK myeloid NGS panels. On retrospective review vacuolisation can be seen throughout his myeloid progenitors. In this case the hallmark features of VEXAS were present, but a lack of disease classification and available UBA1 testing led to delays in diagnosis. The mechanism by which lymphoplasmacytic or plasma cell neoplasms can be complicated with VEXAS syndrome remains unknown and it has not been elucidated whether UBA1 variants are drivers in lymphoplasmacytic conditions.

Conclusion: This case highlights the power of genomic sequencing technologies to identify new genes implicated in inflammatory haematology conditions. Better characterisation of the varied phenotype of VEXAS is needed for disease stratification and the development of targeted treatments, alongside long-term follow-up to compare disease progression in patients with this highly inflammatory microenvironment.

Durvalumab and acalabrutinib for relapsed/refractory (r/r) high-grade B-cell lymphoma: MoST15, open label phase II sub-study of the Molecular Screening and Therapeutics in Leukaemia and Lymphoma (MoST-LLy) framework

Blunt D[1,2], Brown A[3], Dobbins J[3], Amor G[6], Lin F[5], Ross D[1,2], Scott H[3], Lane S[5,6]
[1]Royal Adelaide Hospital, Adelaide, Australia, [2]Haematology Directorate, SA Pathology, Adelaide, Australia, [3]Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, Australia , [4]Kinghorn Centre for Clinical Genomics, University of Sydney, Darlinghurst, Australia, [5]Department of Haematology, Royal Brisbane and Women's Hospital, Brisbane, Australia , [6]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Aim: Patients with high-grade B-cell lymphoma who relapse after frontline immunochemotherapy have a dismal prognosis. Chimeric antigen receptor (CAR) T-cell therapy is a promising treatment. However, with limited availability across Australia further effective regimens with tolerable safety profiles are needed.

Durvalumab is a monoclonal antibody that blocks PD-L1 (programmed cell death ligand-1) which restores T-cell activation and anti-tumour responses[1]. Acalabrutinib, a second generation Bruton Tyrosine Kinase (BTK) inhibitor, blocks BTK which plays an essential role in oncogenic signalling for proliferation and survival in many B-cell malignancies[2]. Previous studies suggest PD-L1 and BTK inhibitors act synergistically[3,4]. The primary objective of this study is to determine the clinical activity of durvalumab and acalabrutinib in 32 patients.

Method: Patients are enrolled through a haematology specific molecular screening MoST-LLy program (ACTRN12616000908437) where tumour genomic profiling is performed using a 523-gene panel (TSO500, Illumina). Durvalumab was administered at 1500mg IV every 28 days and acalabrutinib100mg is given orally twice daily continuously until progression or withdrawal. Exploratory analysis of predictive biomarkers include mutational patterns, tumour mutational burden (TMB), and PD-L1 expression.

Results: As of May 2023, 11 patients received treatment, with a median of 4 prior lines of therapy (range 2-6). Three patients (27%) achieved partial response (according to RECIL criteria) after at least 2 cycles. Mean cycles received was 5 (140 days), with maximum duration on study for 19 cycles. There have been 2 Serious Adverse Events, neither related to study drugs. No patients were withdrawn due to toxicity. TMB at baseline ranged from 6.3-24.6 with a median of 12.9 mutations/megabase. Notably, TP53 mutations were enriched in most non-responding patients. Recruitment and correlative analyses is ongoing.

Conclusion: The combination of durvalumab and acalabrutinib in r/r high-grade B-cell lymphoma was well tolerated in the first 11 patients, showing promising early clinical activity in this heavily pre-treated population.

References:

- Lee HT, Lee JY, Lim H. *et al.* Molecular mechanism of PD-1/PD-L1 blockade via anti-PD-L1 antibodies atezolizumab and durvalumab. *Sci Rep* 7, 5532 (2017).

1) Wu J, Zhang M, Liu D. Acalabrutinib (ACP-196): a selective second-generation BTK inhibitor. *J Hematol Oncol*. 2016 Mar 9;9:21.
2) Herrera AF, Goy A, et al. Safety and activity of ibrutinib in combination with durvalumab in patients with relapsed or refractory follicular lymphoma or diffuse large B-cell lymphoma. Am J Hematol. 2020;95(1):18-27.
3) Younes, Anas et al. Safety and activity of ibrutinib in combination with nivolumab in patients with relapsed non-Hodgkin lymphoma or chronic lymphocytic leukaemia: a phase 1/2a study. The Lancet. Haematology vol. 6,2 (2019): e67-e78. doi:10.1016/S2352-3026(18)30217-5

Pirtobrutinib in covalent BTK-inhibitor pre-treated mantle cell lymphoma: updated results and subgroup analysis from the phase 1/2 BRUIN study with 2 years of survival follow-up

Cheah C[1,2], Shah N[3], Jurczak W[4], Zinzani P[5,6], Eyre T[7], Ujjani C[8], Izutsu K[9], Ma S[10], Flinn I[11], Alencar A[12], Lewis D[13], Patel K[14], Maddocks K[15], Wang Y[16], Munir T[17], Zelenetz A[18], Balbas M[19], Tsai D[19], Wang C[20], Wang M[21]

[1]Linear Clinical Research and Sir Charles Gairdner Hospital, , Australia, [2]Medical School, University of Western Australia, Perth, Australia, [3]Medical College of Wisconsin, , USA, [4]Maria Sklodowska-Curie National Research Institute of Oncology, , Poland, [5]IRCCS Azienda Ospedaliero-Universitaria di Bologna Istituto di Ematologia "Seràgnoli", , Italy, [6]Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale Università di Bologna, , Italy, [7]Oxford University Hospitals NHS Foundation Trust, Churchill Cancer Center, , UK, [8]Fred Hutchinson Cancer Research Center, University of Washington, , USA, [9]Department of Hematology, National Cancer Center Hospital, , Japan, [10]Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine , Chicago, USA, [11]Sarah Cannon Research Institute and Tennessee Oncology, , USA, [12]Sylvester Comprehensive Cancer Center, , USA, [13]Plymouth Hospitals NHS Trust - Derriford Hospital, , UK, [14]Center for Blood Disorders and Cellular Therapy, Swedish Cancer Institute, Seattle, USA, [15]The Ohio State University Comprehensive Cancer Center, , USA, [16]Division of Hematology, Mayo Clinic, Rochester, USA, [17]Department of Haematology, St. James's University Hospital, , UK, [18]Memorial Sloan Kettering Cancer Center, , USA, [19]Loxo@Lilly, , USA, [20]Eli Lilly and Company, , USA, [21]MD Anderson Cancer Center, , USA

Aim: Pirtobrutinib is a highly selective, non-covalent (reversible) BTK-inhibitor (BTKi). We report updated results of pirtobrutinib in patients with cBTKi pre-treated relapsed/refractory (R/R) mantle cell lymphoma (MCL) and >3years follow-up from enrollment.

Method: Patients with cBTKi pre-treated R/R MCL received pirtobrutinib monotherapy in a multicenter phase 1/2 BRUIN trial (NCT03740529). Efficacy was assessed in the prespecified primary efficacy cohort comprising the first 90 enrolled patients who had measurable disease, received prior cBTKi, and no known central-nervous-system involvement. The primary endpoint: overall response rate (ORR), assessed by independent review committee. Secondary endpoints: duration of response (DOR), safety. Data cut: 29-July-2022.

Results: Among MCL patients who received prior cBTKi (n=90), median age was 70years (46-87), median prior lines of therapy were 3 (1-8), 82% discontinued prior cBTKi due to disease progression, 78% had intermediate/high risk sMIPI score. 17/36 (47%) had *TP53* mutations and 25/34 (74%) had Ki67≥30%. ORR was 57% (95%CI, 46-67); 19% complete responses (n=17), 38% partial responses (n=34). Median DOR (median follow-up: 13months) among 51 responding patients was 17.6months (95%CI, 7.3-27.2). 12- and 18-month estimated DOR rates were 58% (95%CI, 41-72) and 45% (95%CI, 27-61). ORR and DOR by subgroups are shown in the Table. The median progression-free survival was 7.4months (95%CI, 5.3–13.3). The median overall survival was 23.5months (95%CI, 15.9-NE). In the MCL safety cohort (n=166), most frequent treatment-emergent adverse events (TEAE): fatigue (31%), diarrhea (22%), anemia (17%). The most common Grade≥3 TEAE was neutropenia (15%). Grade≥3 TEAE of hemorrhage (3%) and atrial fibrillation/flutter (2%) were infrequent. Only 5 (3%) patients discontinued due to a treatment-related AE.

Conclusion: Pirtobrutinib continues to show durable efficacy and favorable safety profile in heavily pre-treated R/R MCL patients with prior cBTKi therapy. Responses were observed in patients with high-risk disease features, including patients with blastoid/pleomorphic variants, elevated Ki67 index, and *TP53* mutations.

Table. ORR and DOR in cBTKi pre-treated MCL pts and high-risk subgroups

		cBTKi pre-Treated MCL, n	ORR, % (95% CI)	DOR, median (95% CI)
Overall		90	56.7 (45.8-67.1)	17.6 (7.3-27.2)
MCL histology	**Classic/Leukemic**	70	58.6 (46.2-70.2)	17.6 (7.5-NE)
	Blastoid	8	50.0 (15.7-84.3)	NE (1.4-NE)
	Pleomorphic	12	50.0 (21.1-78.9)	27.2 (3.7-NE)
***TP53* mutation**	**Yes**	17	47.1 (23.0-72.2)	17.6 (1.7-NE)
	No	19	57.9 (33.5-79.7)	14.8 (1.9-NE)
Ki-67	**<30%**	9	66.7 (29.9-92.5)	17.6 (1.6-NE)
	≥30%	25	56.0 (34.9-75.6)	21.6 (1.7-NE)

Long-term safety with ≥12 months of pirtobrutinib in relapsed/refractory B-cell malignancies

Cheah C[1], Coombs C[2], Shah N[3], Jurczak W[4], Woyach J[5], Patel K[6], Maddocks K[5], Wang Y[7], Muehlenbein C[8], Wang C[9], Abhyankar S[8], Tsai D[8], Eyre T[10]
[1]Linear Clinical Research and Sir Charles Gairdner Hospital, Perth, Australia, [2]UCI Health , Orange, USA, [3]Medical College of Wisconsin, Milwaukee, USA, [4]Maria Sklodowska-Curie National Research Institute of Oncology, Krakow, Poland, [5]The Ohio State University Comprehensive Cancer Center, Columbus, USA, [6]Center for Blood Disorders and Cellular Therapy, Swedish Cancer Institute, Seattle, USA, [7]Division of Hematology, Mayo Clinic, Rochester, USA, [8]Loxo@Lilly, Indianapolis, USA, [9]Eli Lilly and Company, Indianapolis, USA, [10]Oxford University Hospitals NHS Foundation Trust, Churchill Cancer Center, , Oxford, United Kingdom

Aim: Pirtobrutinib is a highly selective, non-covalent (reversible) BTKi approved by the FDA in January 2023 for relapsed/refractory (R/R) mantle cell lymphoma after 2 prior lines of therapy including a Bruton tyrosine kinase inhibitor (BTKi). Pirtobrutinib has promising efficacy with low discontinuation and dose reduction rates in patients (pts) with multiple subtypes of R/R B-cell malignancies. However, the long-term safety and tolerability of pirtobrutinib has not yet been reported. We report clinical safety in pts with long-term (≥12 months) pirtobrutinib treatment from the phase 1/2 BRUIN trial.

Method: Pts with R/R B-cell malignancies who received ≥12 months of pirtobrutinib were included. Median time to onset, dose reduction, discontinuation, and cumulative incidence rates were determined for treatment-emergent adverse event (TEAE) that occurred in ≥20% of pts and select AE of interest associated with BTKi.

Results: As of 29 July 2022, 773 pts were enrolled; 326 (42%) pts received treatment for ≥12 months, among whom median time on treatment was 19 months (IQR: 16,25), with 231 (71%) remaining on pirtobrutinib. The most common TEAE (all-grade, regardless of attribution) were fatigue (32%), diarrhea (31%), Covid-19 (29%), contusion (26%), cough (25%), back pain (21%). TEAE leading to dose reduction or discontinuation occurred in 23 (7%) and 11 (3%) pts. Four (1%) pts discontinued due to a treatment-related AE, and 1 pt had a fatal treatment-related AE (Covid-19 pneumonia). Select AE of interest for long-term pts are shown in the Table. Comprehensive safety analyses describing the frequency of TEAE over time will be presented.

Conclusion: Prolonged pirtobrutinib therapy continues to demonstrate a safety profile amenable to long-term administration at the recommended dose without evidence of new or worsening toxicity signals. Safety and tolerability observed in pts on therapy for ≥12 months was similar to previously published safety analyses on all pts enrolled, regardless of follow-up.

Select AE of interest associated with BTKi in pts with ≥12 months exposure (N=326)

AE	Any-Grade TEAE %	Grade ≥3 TEAE %	Median time (months) to first occurrence (Q1, Q3)	Leading to dose reduction %	Leading to drug discontinuation %	Cumulative incidence rate (6, 12, 24 months) %
Bruising[a]	31	0	1.8 (0.5, 5.6)	<1	0	23, 27, 29
Arthralgia	21	1	7.4 (2.9, 12.0)	0	0	9, 16, 20
Rash[a]	20	<1	2.4 (0.7, 9.1)	0	0	13, 15, 18
Hemorrhage/ Hematoma[a]	17	2	5.8 (1.9, 13.7)	0	0	9, 11, 16
Hypertension	16	3	6.9 (2.1, 11.6)	<1	0	7, 12, 16
Atrial fibrillation/ flutter[a]	3	1	10.2 (3.8, 15.0)	0	0	1, 2, 2

[a]Consolidated Terms.

BRUIN MCL-321: A phase 3, open-label, randomized study of pirtobrutinib versus investigator choice of BTK inhibitor in patients with previously treated, BTK inhibitor-naïve mantle cell lymphoma (trial in progress)

Cheah C[1], Wang M[2], Eyre T[3], Shah N[4], LeGouill S[5], Dreyling M[6], Vandenberghe E[7], Wang Y[8], Song Y[9], Gandhi M[10], Chay C[11], Sharman J[12], Andorsky D[13], Ruppert A[14], Duann C[15], Muthig V[15], Ito R[15], Jurczak W[16]

[1]Linear Clinical Research and Sir Charles Gairdner Hospital, , Australia, [2]University of Texas MD Anderson Cancer Center, Houston, USA, [3]Oxford University Hospitals NHS Foundation Trust, Churchill Cancer Center, , United Kingdom, [4]Medical College of Wisconsin, Milwaukee, USA, [5]Institut Curie, Paris, France, [6]Department of Medicine III, LMU University Hospital, Munich, Germany, [7]Hope Directorate St. James Hospital, Dublin, Ireland, [8]Division of Hematology, Mayo Clinic, Rochester, USA , [9]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Beijing, China, [10]Virginia Cancer Specialists, Fairfax, USA, [11]Messino Cancer Centers, Asheville, USA, [12]Willamette Valley Cancer Institute and Research Center, US Oncology Research, Eugene, USA, [13]Rocky Mountain Cancer Centers, US Oncology Research, Boulder, USA, [14]Eli Lilly and Company, Indianapolis, USA, [15]Loxo@Lilly, Indianapolis, USA, [16]Maria Sklodowska-Curie National Research Institute of Oncology, , Poland

Aim: Covalent (c) Bruton tyrosine kinase inhibitors (BTKi) have transformed management of MCL, but are not curative, sharing pharmacologic liabilities (low oral bioavailability, short half-life), which may lead to suboptimal BTK target coverage, especially in rapid-proliferating tumors with high BTK protein turnover like MCL, which can manifest as acquired resistance to BTKi. Pirtobrutinib, a highly selective, non-covalent (reversible) BTKi, has favorable oral pharmacology enabling continuous BTK inhibition throughout the dosing interval, regardless of intrinsic BTK turnover rate. In phase1/2 BRUIN, pirtobrutinib demonstrated promising durable overall response rates and was well tolerated in patients with MCL regardless of prior therapy (including cBTKi), number of prior lines of therapy, or reason for prior cBTKi discontinuation. We aim to establish whether pirtobrutinib is superior to investigator's choice of cBTKi in patients with previously treated, BTKi-naïve MCL.

Method: BRUIN MCL-321 (NCT04662255) is a randomized, open-label, global phase3 study comparing pirtobrutinib monotherapy versus ibrutinib/acalabrutinib/zanubrutinib. ~500 patients will be randomized 1:1 and stratified by sMIPI risk (low/intermediate vs high), comparator BTKi (ibrutinib vs acalabrutinib/zanubrutinib), and number of prior lines of therapy (1 vs ≥2). Enrollment is ongoing.

Adults with MCL who received ≥1 prior line of systemic therapy and are BTKi-naïve are eligible. Patients must have measurable disease by imaging per Lugano criteria and have progressed on or relapsed following the most recent line of therapy. History of current/prior CNS involvement, significant cardiovascular disease, stroke/intracranial hemorrhage within 6months of randomization, and autologous stem cell transplant (SCT)/allogeneic SCT/chimeric antigen receptor T-cell therapy within 60days of randomization are key exclusion criteria.

Progression-free survival (PFS) per Lugano criteria assessed by an independent review committee is the primary endpoint. Overall response rate, duration of response, investigator-assessed PFS per Lugano criteria, overall survival, event-free survival, time-to-treatment failure, time-to-next treatment, PFS2, safety and tolerability, and patient-reported outcomes are secondary endpoints.

ZUMA-23: a global, phase 3, randomized controlled study of axicabtagene ciloleucel versus standard of care as first-line therapy in patients with high-risk large B-cell lymphoma.

Dickinson M[1], Westin J[2], Jacobson C[3], Chavez J[4], Sureda A[5], Morschhauser F[6], Glaß B[7], Davies A[8], Flinn I[9], Maloney D[10], Chamuleau M[11], Tees M[12], Xue A[13], Shahani S[13], Nikolajeva O[13], Kang J[13], Kaplan A[13], Schupp M[13], Miao H[13], Shima Rich E[13]

[1]Peter Maccallum Cancer Centre, Royal Melbourne Hospital And The University Of Melbourne, Melbourne , Australia, [2]The University of Texas MD Anderson Cancer Center, Houston, USA, [3]Dana-Farber Cancer Institute, Boston, USA, [4]Moffitt Cancer Center, Tampa, USA, [5]Hematology Department, Institut Català d'Oncologia-Hospitalet, Barcelona, Spain, [6]University of Lille, CHU Lille, Lille, France, [7]Helios Klinikum Berlin-Buch, Berlin, Germany, [8]Southampton Cancer Research UK/NIHR Experimental Cancer Medicines Centre, University of Southampton, Southampton, UK, [9]Sarah Cannon Research Institute and Tennessee Oncology, Nashville, USA, [10]Fred Hutchinson Cancer Center, Seattle, USA, [11]Amsterdam University Medical Center, Amsterdam, Netherlands, [12]Colorado Blood Cancer Institute, Denver, USA, [13]Kite, a Gilead Company, Santa Monica, USA

Aim: The nearly 40% of patients with large B-cell lymphoma (LBCL) who are refractory to or relapse after first-line standard-of-care (SOC) regimens, such as R-CHOP and DA-EPOCH-R, have poor prognoses. High International Prognostic Index (IPI) score and the subtype of high-grade B-cell lymphoma (HGBL) are associated with shorter progression-free and overall survival (PFS and OS; Nastoupil LJ and Bartlett NL. *J Clin Oncol.* 2023).

Axicabtagene ciloleucel (axi-cel) is an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy approved to treat relapsed/refractory (R/R) LBCL. In the Phase 2 ZUMA-12 study in first-line LBCL, axi-cel showed an objective response rate of 89% (complete response rate, 78%; median follow-up, 15.9 months; Neelapu SS, et al. *Nat Med.* 2022). ZUMA-23 is the first Phase 3, randomized controlled study to evaluate CAR T-cell therapy as first-line regimen for any cancer and will assess axi-cel versus SOC in patients with high-risk LBCL, defined as IPI 4-5.

Method: The Phase 3 trial design will enroll ≈300 adults with high-risk LBCL, including diffuse LBCL (DLBCL), HGBL, and transformed follicular or marginal zone lymphoma. Eligible patients will receive 1 cycle of R-chemotherapy and then be randomized 1:1 to receive axi-cel or SOC. Patients in the axi-cel arm will undergo leukapheresis, then receive R-CHOP or DA-EPOCH-R bridging therapy, followed by lymphodepleting chemotherapy, and axi-cel infusion (2×10^6 CAR T cells/kg). Prophylactic corticosteroids may be administered at investigator's discretion. Patients in the SOC arm will receive 5 additional cycles of R-CHOP or DA-EPOCH-R.

The primary endpoint is event-free survival by blinded central review. Key secondary endpoints are OS and PFS. Safety, quality of life, and pharmacokinetics will also be assessed. Patients with a history of HIV and/or hepatitis B or C and undetectable viral loads may enroll. Key exclusion criteria include LBCL of the central nervous system. ZUMA-23 is open for enrollment (NCT05605899).

Primary overall survival analysis of the phase 3 randomised Zuma 7 study of axicabtagene ciloleucel versus standard of care therapy in relapsed/refractory large B-cell lymphoma

Dickinson M[1], Westin J[2], Oluwole O[3], Kersten M[4], Miklos D[5], Perales M[6], Ghobadi A[7], Rapoport A[8], Sureda A[9], Jacobson C[10], Farooq U[11], van Meerten T[12], Ulrickson M[13], Elsawy M[14], Leslie L[15], Chaganti S[16], Yang Y[17], Schupp M[17], To C[17], Locke F[18]

[1]Peter Maccallum Cancer Centre, Royal Melbourne Hospital And The University Of Melbourne, Melbourne , Australia, [2]The University of Texas MD Anderson Cancer Center, Houston, USA, [3]Vanderbilt-Ingram Cancer Center, Nashville, USA, [4]Amsterdam UMC, University of Amsterdam, Cancer Center Amsterdam, Amsterdam, The Netherlands, [5]Stanford University School of Medicine, Stanford, USA, [6]Memorial Sloan Kettering Cancer Center, New York, USA, [7]Washington University School of Medicine, St. Louis, USA, [8]The Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, USA, [9]Hematology Department, Institut Català d'Oncologia-Hospitalet, Barcelona, Spain, [10]Dana-Farber Cancer Institute, Boston, USA, [11]University of Iowa, Iowa City, USA, [12]University Medical Center Groningen, Groningen, The Netherlands, [13]Banner MD Anderson Cancer Center, Gilbert, USA, [14]Division of Hematology and Hematologic Oncology, Department of Medicine, Dalhousie University and QEII Health Sciences Center, Halifax, Canada, [15]John Theurer Cancer Center, Hackensack, USA, [16]Centre for Clinical Haematology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK, [17]Kite, a Gilead Company, Santa Monica, USA, [18]Moffitt Cancer Center, Tampa, USA

Aim: To report the primary overall survival (OS) analysis of ZUMA-7, an international phase 3 trial comparing axicabtagene ciloleucel (axi-cel) versus standard-of-care therapy (SOC; 2-3 cycles of chemoimmunotherapy followed in responding patients by high-dose chemotherapy with autologous stem-cell transplantation) as second-line treatment for relapsed/refractory large B-cell lymphoma (LBCL).

Method: Study procedures and eligibility were previously reported (Locke, et al. *NEJM*. 2022). The intention-to-treat primary OS analysis occurred 5 years after the first patient was randomized (01/25/2018) per protocol. A log-rank test stratified by randomization stratification factors compared OS between the 2 arms. Other endpoints included progression-free survival (PFS) by investigator, OS in key prespecified subgroups, and safety.

Results: In total, 359 patients were randomized, 180 to axi-cel and 179 to SOC. As of 01/25/2023, at a median follow-up of 47.2 months, axi-cel demonstrated a statistically significant improvement in OS over SOC (HR [95% CI], 0.726 [0.540-0.977]; stratified log-rank 1-sided *P*=0.0168 [efficacy boundary, 0.0249]). This increased survival with axi-cel was observed in the intention-to-treat population, which included 74% with primary refractory disease and other high-risk features. Median OS was longer with axi-cel versus SOC (not reached versus 31.1 months, respectively); 48-month OS estimates were higher with axi-cel (54.6% versus 46.0%, respectively). OS benefit with axi-cel versus SOC was similar in key prespecified subgroups, including age ≥65 years, primary refractory, early relapse, high-grade B-cell lymphoma, and high second-line age-adjusted International Prognostic Index. In the SOC arm, 102 patients (57%) received subsequent cellular immunotherapy off protocol. PFS by investigator confirmed benefit of axi-cel over SOC (HR [95% CI], 0.506 [0.383-0.669]), with 48-month PFS estimates of 41.8% versus 24.4%, respectively. No new treatment-related deaths occurred since the primary event-free survival analysis.

Conclusion: Axi-cel as second-line therapy for early relapsed/refractory LBCL significantly improved OS versus SOC.

Progressive Multifocal Leukoencephalopathy and Lymphoma - Pembrolizumab May Be Beneficial

D'Souza M[1], Ayyalil F[1], Zhai S[2], Ong C[2]
[1]The Canberra Hospital, Garren, Australia, [2]Calvary Hospital, Bruce, Australia

Progressive Multifocal Leukoencephalopathy (PML) is a rare, fatal demyelinating disease of the central nervous system. Reactivation of the John Cunningham (JC) virus in immunocompromised individuals may lead to PML. The association between PML and immunosuppression related to haematological malignancies and treatment has been well established. There is recent interest in the use of immune checkpoint inhibitors to target programmed cell death protein 1 (PD-1) to manage PML. We compare two cases of PML in patients with an underlying haematological malignancy, with two clinically distinct outcomes.

A 73-year-old man with Stage IV Classical Hodgkin's lymphoma was treated with doxorubicin/ bleomycin/ vinblastine/ dacarbazine (ABVD) chemotherapy. Following three cycles of treatment, he presented with subacute confusion and altered behaviour. He was diagnosed with PML based on the detection of JC virus DNA in cerebrospinal fluid (CSF) and compatible MRI brain lesions. He concurrently developed pneumocystis jiroveci pneumonia (PJP) and pulmonary cryptococcosis, that was managed with high dose trimethoprim/ sulfamethoxazole and fluconazole. Interval progression of PML was treated with a trial of pembrolizumab, leading to clinical and radiological improvement, along with a reasonable improvement in quality of life.

A 78-year-old woman with transformed diffuse large B-cell lymphoma (DLBCL) completed 6 cycles of obinutuzumab/ cyclophosphamide/ doxorubicin/ vincristine/ prednisolone (O-CHOP) followed by obinutuzumab maintenance. She presented with word-finding difficulty and memory loss and was subsequently diagnosed with PML. Immunosuppression was ceased. Pembrolizumab was not offered due to treatment preference at that time and medication access issues. She suffered significant neurological decline within few months of diagnosis.

These two cases highlight that PML should be considered in patients with haematological malignancies presenting with neurological symptoms. Pembrolizumab could be explored as a possible treatment option for PML. Further studies are warranted to determine role and treatment benefits of checkpoint inhibitors in PML.

Phase 1b/2a study of AZD4573 (CDK9i) and acalabrutinib in patients (pts) with relapsed/refractory diffuse large B-cell lymphoma (r/r DLBCL)

Danilov A[1], **Gregory G[2]**, Morschhauser F[3], Cheah C[4], Shah H[5], Jurczak W[6], Olabode D[7], Meyer S[8], Yoon J[8], Arduini S[9], Saeh J[8], Olsson R[10], Strati P[11]

[1]City of Hope Comprehensive Cancer Center, Duarte, USA, [2]Clinical Sciences at Monash Health, Monash University, Melbourne, Australia, [3]CHRU Lille - Hopital Claude Huriez, Lille Cedex, France, [4]Sir Charles Gairdner Hospital and University of Western Australia, Perth, Australia, [5]Huntsman Cancer Center, University of Utah, Salt Lake City, USA, [6]Maria Sklodowska-Curie National Research Institute of Oncology, Krakow, Poland, [7]AstraZeneca, Waltham, USA, [8]AstraZeneca, Boston, USA, [9]AstraZeneca, Cambridge, UK, [10]AstraZeneca, Gothenburg, Sweden, [11]The University of Texas MD Anderson Cancer Center, Houston, USA

Aim: Here we report pooled data from the completed Phase 1b and ongoing Phase 2a expansion.

Methods: Pts were ≥18 years old with ECOG PS ≤2, and had received ≥2 prior lines of therapy (stem cell transplant, T cell engagers [TCEs] and/or CAR-T were allowed). Following a 3-wk intra-pt ramp-up, pts received AZD4573 QW of 9 mg or 12 mg. Objectives included ORR, safety and PK. Responses were assessed by Lugano 2014 criteria and adverse events (AEs) graded using CTCAE v5.0.

Results: As of 2 December, 2022, 27 pts were treated (11 pts in escalation, 16 pts in expansion;). Median age was 60 yrs and median number of prior lines of treatment was 4. Median duration of AZD4573 treatment was 14.9 wks and 8.9 wks. Response was evaluable in 24 pts; 11/24 had prior CAR-T and 5/24 had prior TCE. The ORR was 50.0% (95% CI 29.1, 70.9) and the CR rate was 25%.. Median duration of response was 6.8 mos. Responses were noted in 5/11 pts with prior CAR-T (ORR 45.5%; 95% CI 16.7, 76.6. Responses were seen in both GCB (7 evaluable pts; 2 CRs/1 PR) and non-GCB (8 evaluable pts; 2 CRs/2 PRs) subtypes. No DLTs were identified and both doses were expanded. Safety was evaluable in 27 pts. The most common TEAEs were primarily laboratory-based. ALT/AST and bilirubin increases were mainly due to down-modulation of hepatic transporter proteins and reduced enzyme clearance, not direct hepatocellular injury; all were short-lived with spontaneous resolution and caused no treatment delays. Neutropenia occurred in 92.6% of pts but was manageable with G-CSF; 4/27 pts (14.8%) had Grade ≥3 infections. One pt discontinued due to AZD4573-related Grade 2 fatigue.

Conclusions: AZD4573 + acalabrutinib had manageable safety with no new signals. Clinical activity was promising with durable responses in heavily pretreated pts, including those with prior CAR-T. More mature data will be presented at the conference. EA - previously submitted to EHA 2023.

Phase 3 trial of subcutaneous epcoritamab + R-CHOP versus R-CHOP in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL): EPCORE DLBCL-2

Sehn L[1], Chamuleau M[2], Lenz G[3], Clausen M[4], Haioun C[5], Izutsu K[6], Davies A[7], Zhu J[8], Oki T[9], Szafer-Glusman E[9], Conlon R[9], Chiou H[9], Ipe D[9], Elliott B[10], Wu J[9], **Gregory G[11]**, Salles G[12]
[1]BC Cancer Centre for Lymphoid Cancer and the University of British Columbia, Vancouver, Canada, [2]Amsterdam UMC, Vrije Universiteit, Amsterdam, Netherlands, [3]Department of Medicine A for Hematology, Oncology and Pneumology, Universitätsklinikum Münster, Münster, Germany, [4]Sygehus Lillebælt, Vejle, Denmark, [5]Hopital Henri Mondor, Créteil, France, [6]National Cancer Center Hospital, Tokyo, Japan, [7]Southampton NIHR/Cancer Research UK Experimental Cancer Medicines Centre, University of Southampton, Southampton, United Kingdom, [8]Beijing Cancer Hospital, Beijing, China, [9]AbbVie, North Chicago, USA, [10]Genmab, Plainsboro, USA, [11]Monash University, Melbourne, Australia, [12]Memorial Sloan Kettering Cancer Center, New York, USA

Aim: Epcoritamab, a subcutaneous CD3xCD20 bispecific antibody, demonstrated deep and durable responses (ORR, 63%; CR, 39%) with a manageable safety profile in patients with R/R aggressive large B-cell lymphoma (LBCL). Epcoritamab received FDA approval for adults with R/R DLBCL not otherwise specified (NOS), including DLBCL arising from indolent lymphoma, and high-grade B-cell lymphoma (HGBCL) after ≥2L of systemic therapy. In the ongoing phase 1/2 EPCORE NHL-2 (NCT04663347), epcoritamab+R-CHOP showed promising efficacy and manageable safety in patients with newly diagnosed DLBCL with International Prognostic Index (IPI) 3-5 (n=46; ORR: 100%; complete metabolic response [CMR]: 76%). Cytokine release syndrome (CRS) events were primarily low-grade (57% G1-2; 2% G3) and had predictable timing; all cases resolved (Falchi et al, ASCO 2023, abstract 7519). These encouraging data support further evaluation of 1L epcoritamab+R-CHOP.

Method: This phase 3, global, multicenter, open-label study (NCT05578976) evaluates efficacy and safety of epcoritamab+R-CHOP in adults newly diagnosed with one of the following CD20+ DLBCL (de novo or transformed from follicular lymphoma [FL]): 1) DLBCL, NOS; 2) HGBCL with MYC and BCL-2 and/or BCL-6 rearrangement; 3) T-cell/histiocyte-rich LBCL; 4) EBV+ DLBCL, NOS; or 5) FL grade 3b. Other key eligibility criteria include IPI ≥2 (IPI 2 not to exceed ~30% of patients), ECOG PS 0-2, and ≥1 measurable disease site. ~900 patients will be randomized 2:1 to epcoritamab+R-CHOP (6 cycles, followed by 2 cycles of epcoritamab) or R-CHOP (6 cycles, followed by 2 cycles of rituximab). The primary efficacy endpoint is progression-free survival (PFS) in patients with IPI 3-5 (IRC-assessed per Lugano criteria). Secondary efficacy endpoints include PFS in patients with IPI 2-5, event-free survival, CMR, overall survival, and minimal residual disease negativity. Safety endpoints include incidence/severity of treatment-emergent adverse events (AEs), serious AEs, and AEs of special interest (CRS, immune cell-associated neurotoxicity syndrome, clinical tumor lysis syndrome). Enrollment began January 2023.

Results: N/A

Conclusion: N/A

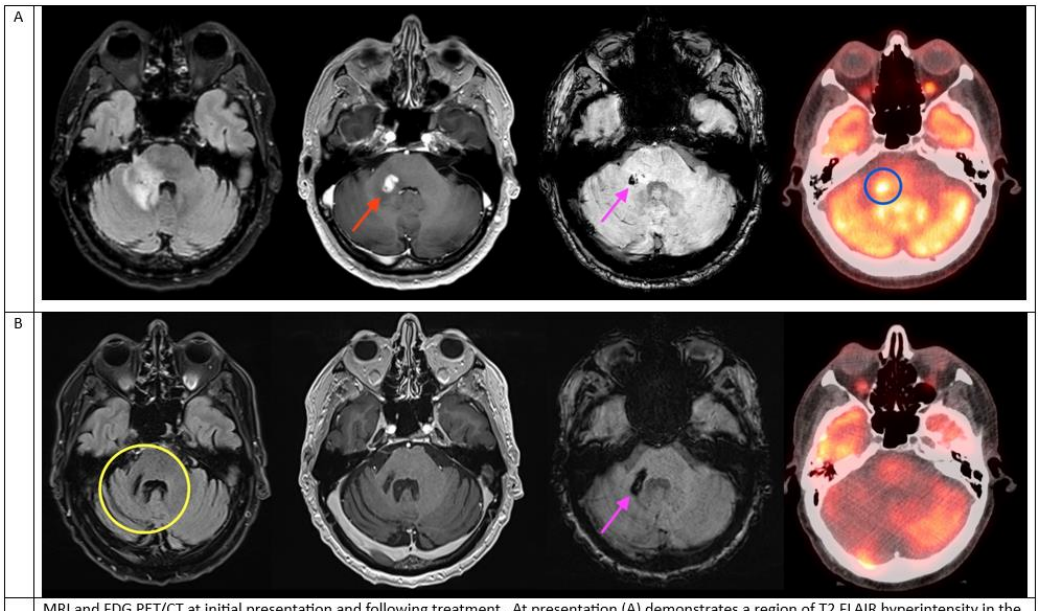
Primary central nervous system (CNS) T-cell lymphoma as the presenting manifestation of late–onset combined immunodeficiency (LOCID)

Jeffrey A[1,2], Coyle L[1,2], Samaranayake D[1], Boyle T[3,4], Drummond J[5], Fernando S[2,3,4]
[1]Department of Haematology, Royal North Shore Hospital, St Leonards, Australia, [2]Northern Clinical School, The University of Sydney, Sydney, Australia , [3]Department of Clinical Immunology and Allergy, Royal North Shore Hospital, St Leonards, Australia, [4]Immunology Laboratory, Royal North Shore Hospital, St Leonards, Australia , [5]Department of Radiology, Royal North Shore Hospital, St Leonards, Australia

Background: Late-onset combined immunodeficiency (LOCID), considered now a subset of common variable immunodeficiency (CVID) disorders is characterized by a predominantly T-cell immune defect. LOCID has a distinct phenotype from CVID with a greater risk of lymphoproliferative complications. As compared to the CVID cohort LOCID patients also have increased rates of splenomegaly and granulomatous disease. This is the first reported case of T cell lymphoma in an adult patient with LOCID.

Case Presentation: A 67 year old male, presented with a 4 week history of sensory disturbance and ataxia. MRI brain identified an enhancing lesion within the cerebellum. Brain biopsy demonstrated a histiocyte rich T-cell lymphoma consistent with a WHO diagnosis of peripheral T cell lymphoma, NOS. EBER-ISH was negative. Investigations to evaluate immune function demonstrated panhypogammaglobulinaemia with T and B cell lymphopenia, absent haemoglutinins and impaired functional antibody responses. HIV serology was negative. The patient achieved a complete response to therapy after 4 cycles of MATRix (methotrexate, cytarabine, thiotepa) and 2 cycles of ICE (etoposide, carboplatin, ifosfamide) chemotherapy followed by CNS directed autologous stem cell transplantation with carmustine and thiotepa conditioning. Intravenous immunoglobulin replacement was commenced to address the underlying immunodeficiency. FDG-avid pulmonary lesions consistent with a diagnosis of Granulomatous and Lymphocytic Intersititial Lung Disease (GLILD) were identified as a second non-infectious complication of LOCID. The pulmonary lesions resolved after chemotherapy and immunoglobulin replacement. The patient remains well with no evidence of disease recurrence now more than 18 months after completion of therapy.

Conclusion: This case raises awareness of T-cell lymphoproliferative disorders as a manifestation of CVID-disorders including LOCID. Further study is needed to elucidate the mechanisms of transformation of B or T-cells to lymphoproliferation in primary immunodeficiency patients and define evidence based therapeutic strategies for this challenging cohort of patients.



MRI and FDG PET/CT at initial presentation and following treatment. At presentation (A) demonstrates a region of T2 FLAIR hyperintensity in the right middle cerebellar peduncle with an avidly enhancing 13mm nodule (red arrow) on post contrast T1. There was evidence of a small focus of intralesional haemorrhage on susceptibility weighted imaging (pink arrow). The lesion demonstrates moderately avid tracer uptake SUVMax 7.6 Following biopsy, chemotherapy and ASCT (B) there was complete resolution of the T2 FLAIR signal (yellow ring), no residual enhancement on post contrast T1 and haemosiderin staining (pink arrow). No residual FDG tracer uptake within this focus on FDG-PET.

Mature B-cell lymphoma presenting as a leukoerythroblastic bicytopenia and diagnostic dilemma due to isolated marrow involvement with associated fibrosis: a case report

Kennedy A[1], Swain F[1], Handunnetti S[1], Batt T[1], Norris D[1]
[1]*Princess Alexandra Hospital, Woolloongabba, Australia*

A 49 year old female presented with anaemia, thrombocytopenia and a leucoerythroblastic blood film. She had no constitutional symptoms nor palpable lymphadenopathy or hepatosplenomegaly. Her medical history was significant for hyperlipidemia. She had a family history of malignancy.

Haemoglobin was 100g/L, platelet count $38x10^9$/L with white cell count $9.5x10^9$/L. Blood film demonstrated teardrop cells. LDH was 425U/L. Bone marrow aspirate demonstrated an aparticulate haemodilute aspirate with no clonal population on flow cytometry. Trephine biopsy demonstrated normocellular but disorganised haematopoiesis, with markedly increased fibrosis and an abnormal moderate infiltrate of mature CD20+ cells. Relevant negative results included: Jak2 V617F polymerase chain reaction (PCR); fluorescent in-situ hybridisation (FISH) for *BCR::ABL*, *IGH::BCL2*, *CCND::IGH* and *IGH* breakapart breakapart probes; and CD5/CD10/CD30 and light chain immunohistochemistry. Targeted next generation sequencing panel performed on DNA extracted from bone marrow aspirate demonstrated an assumed somatic origin TET2 c1576C>T variant with a variant allele frequency (VAF) of 3% and a PDCD1LG2 c689G>A variant of uncertain origin with a VAF of 50%. Hair samples have been processed for germline testing (pending). CT imaging of neck/chest/abdomen/pelvis did not identify lymphadenopathy or splenomegaly. PET/CT demonstrated only an FDG-avid scalp lesion; subsequently determined to be an incidental cyst.

Her case was reviewed by an external expert who reported that the monomorphic B-cell infiltrate, with an interstitial distribution and expansion between and around fat spaces with associated reticulin increase was consistent with a B-cell lymphoproliferative disorder, favouring splenic marginal zone lymphoma. The TET2 variant is favoured to be clonal haematopoiesis of indeterminate potential (CHIP); the PD-L2 variant is suspected to be a germline variant of uncertain significance (pending confirmatory germline testing). She has commenced R-CHOP therapy.

This case highlights the ongoing critical role of morphology in the diagnosis of haematological malignancies despite advances in ancillary testing including next generation sequencing panels.

Leukaemic Non-Nodal Mantle Cell Lymphoma: A case study with literature review

Lacey C[1], Chen S[1], Wong C[1]
[1]*Australian Clinical Labs, Clayton, Australia*

Background: Mantle cell leukaemia (MCL) is an aggressive non-Hodgkin lymphoma arising from lymphocytes in the mantle zone of the lymph nodes. It is defined by a somatic mutation and overexpression of cyclin D1 due to translocation of chromosome 11 and 14 t(11;14)(q13;q32). Accurate diagnosis sometimes can be challenging due to multiple morphological variants and atypical pathological features. Genetic testing for t(11;14) and its surrogate marker of Cyclin D1 on the immunohistochemistry are critical for a definitive diagnosis.

Aim: To discuss a case of MCL - leukaemic non-nodal (L-NN) MCL and to present currrent research on this disease.

Method: PubMed was searched using the term “Mantle cell lymphoma”. The search was refined based on year of publication to the last 10 years and English language. Results were further filtered after review of title and abstracts. The search yielded 19 articles for full text review and inclusion in discussion.

Results: A 53-year-old patient presented with splenomegaly and abnormal lymphocytes in the peripheral blood film. The lymphocytes were medium in size with immature chromatin and single nucleolus. Patient had no detectable lymphadenopathy and otherwise normal counts on the full blood examination. Immunophenotype revealed a monoclonal B-cell population which was CD5-, CD10-, CD19+, CD20+, CD23+, FMC7+ and Lambda sIg+. Bone marrow biopsy detected several small interstitial lymphoid aggregates which were positive for Cyclin D1. Cytogenetics and Molecular studies were performed and showed an unbalanced 11;14 rearrangement and complex karyotype with deletion of 17p. The diagnosis of MCL was made, specifically, leukaemic non-nodal MCL.

Conclusion: Our understanding of atypical and rare forms of MCL is growing. Cyclin D1 on immunohistochemistry for all low-grade lymphomas should be considered as cytogenetic test may be negative for cases with low level of marrow involvement. Accurate diagnosis of MCL can allow for prognostication and for appropriate treatment options.

Hepatosplenic T-cell lymphoma treated with platinum-based induction chemotherapy followed by myeloablative allogeneic stem cell transplant

Lavrukhina H[1], Morris K
[1]*Queensland Health, Brisbane , Australia*

Hepatosplenic T-cell lymphoma (HSTCL) is a rare extranodal T-cell malignancy that classically occurs in younger immunosuppressed males. It is rapidly progressive and has a poor prognosis when treated with conventional CHOP-based chemotherapy, with a reported response duration of 8 months and median overall survival of 16 months. Evaluating the efficacy of induction regimens is difficult due to the rare nature of the disease and hence inability to perform randomised control trials.

This is a case report of an immunocompetent 32 year old male who presented with nausea and vomiting, weight loss and left upper quadrant pain with massive splenomegaly (22.4cm). Bone marrow biopsy showed infiltration by a mature CD3+ T-cell population expressing gamma/delta TCR that was negative for CD4, CD8, CD56, EBER and alpha/beta TCR. Karyotype was normal and FISH for 7q34 abnormalities showed a normal pattern. TCR rearrangement studies showed a monoclonal in polyclonal pattern for TCRG and TCRB.

The patient underwent induction treatment with ICE (ifosfamide, carboplatin, etoposide) given on a conventional 3 day schedule. After 2 cycles he achieved symptomatic improvement with resolution of splenomegaly and clearance of bone marrow disease. At the time of writing he is being prepared for a consolidative allogeneic stem cell transplant using an HLA-identical unrelated donor.

Flow cytometric immunophenotype of CAR T-cells in pleural fluid and differences with peripheral blood CAR T-cells in a patient with Large B-Cell Lymphoma.

Li E[1], Aklilu E[1], Potter A[1], Yang [1], Debosz K[1], Anderson E[1], Ho J[1], Vanguru V[1], Abadir E[1]
[1]*Royal Prince Alfred Hospital, Camperdown, Australia*

Case Report: Chimeric antigen receptor T-cell therapy (CAR-T) is effective in CD19+ malignancies such as DLBCL. Assessing CAR T-cell immune profile following therapy is desirable and may have prognostic significance.

A 56-year-old man with DLBCL with pleural involvement refractory to second line chemo-immunotherapy received axicabtagene ciloleucil. He developed cytokine release syndrome requiring supplemental oxygen. Chest CT revealed a moderate left-sided effusion, and the pleural fluid(PF) was analysed along with paired peripheral blood(PB) sample. Flow cytometry panel was designed to detect commercial anti-CD19-CARs and included PD-1 and TIGIT as T-cell inhibitory and CD69 as a T-cell activation biomarkers.

In the PB, total CAR T-cell count was 160.5x10^6/uL. In PF the CD8+ CAR T-cells had increased TIGIT and CD69 but similar PD-1 expression relative to the PF CD4+ CAR T-cells (c.f. green peaks Figure 1A vs 1B). CD8+ CAR T-cells of PF and PB revealed a significantly increased CD69 expression in PF(c.f. green to pink peak in Figure 1A). In contrast, in the CD4+ CAR T-cell population, there were no significant in biomarker expression between the PB and PF(c.f. Figure 1B green vs pink peaks).

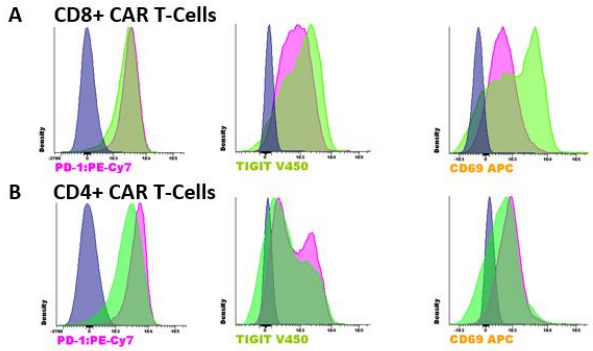


Figure 1. Fluorescence expression of PD-1, TGIT and CD69 on CD8+ (Figure 1A) and CD4+(Figure 1B) CAR T-cells from PF (green peak), and PB (pink peak). Background fluorescence from combined unstained CAR T-cells (FMO) pleural fluid (blue peak).

This is the first case report describing the immunophenotypic profile of CAR T-cells in PF with unexpected difference in activation markers compared to PB CAR T-cells. The higher expression of CD69, a marker of early leukocyte activation and required for establishing tissue inflammation and tumour infiltration(1-3), is noteworthy. Further research should be directed on investigating its significance, and caution is advised on analysing PB CAR T-cell alone as they may not be representative its immunologic profile at the site of disease.

1. Ziegler SF, Ramsdell F, Hjerrild KA, Armitage RJ, Grabstein KH, Hennen KB, et al. Molecular characterization of the early activation antigen CD69: a type II membrane glycoprotein related to a family of natural killer cell activation antigens. Eur J Immunol. 1993;23(7):1643-8.
2. Mita Y, Kimura MY, Hayashizaki K, Koyama-Nasu R, Ito T, Motohashi S, et al. Crucial role of CD69 in anti-tumor immunity through regulating the exhaustion of tumor-infiltrating T cells. International Immunology. 2018;30(12):559-67.
3. Vuckovic S, Bryant CE, Lau KHA, Yang S, Favaloro J, McGuire HM, et al. Inverse relationship between oligoclonal expanded CD69- TTE and CD69+ TTE cells in bone marrow of multiple myeloma patients. Blood Adv. 2020;4(19):4593-604.

Demographic, Clinical Characteristics and Survival Outcomes of the South Auckland Cohort of Primary Central Nervous System Lymphoma

Li J[1]
[1]*Te Whatu Ora, Auckland, New Zealand*

Aim: Middlemore Lymphoma Registry was established to prospectively collect epidemiological and clinical information on all patients with a new diagnosis of non-Hodgkin lymphoma in Counties Manukau since 2006. We aimed to describe their baseline demographic data, clinical characteristics, including prognostic scores and treatment response, and survival outcomes.

Method: This study retrospectively reviewed the Middlemore Lymphoma Registry data on all adult patients (aged ≥ 18 years) with a new primary central nervous system lymphoma diagnosis from 2006–2022. Electronic medical records were accessed for age, gender, ethnicity, initial response to treatment, choice of chemotherapy, the date of histological diagnosis, death, or last follow-up.

Results: A total of 40 patients were identified and accounted for 3.6% of all non-Hodgkin lymphomas. Our cohort had a median age of 64 years (range 18-79 years), male predominant distribution (62.5%), and 25% of patients self-identifying as New Zealand Māori or Pacific Island ethnicities. Comorbidities were present at the initial diagnosis of 27.5% of patients. The majority did not experience B symptoms (90%). Approximately half of our cohort had intermediate-risk disease based on the International Extranodal Lymphoma Study Group prognostic score of 2-3, while 25% had the high-risk disease (scoring 4-5). Most patients (92%) received induction chemoimmunotherapy, and 70% achieved either complete remission or complete remission unconfirmed. The median overall survival was 20 months.

Conclusion: This retrospective review described the demographic and clinical characteristics of the South Auckland cohort with primary central nervous system lymphoma. There was no statistical difference in survival outcomes between different ethnicities.

Monitoring for CAR-T Cell Therapy Related Neurotoxicity in a Visually Impaired Patient

Li N[1], Wallach E[1], Looi W[1], Abbott C[1], Griffith S[1], Kazzi C[1], Inam S[1], Monif M[1], Rahman M[1], Seery N[1], Tam C[1], Wesselingh R[1], Wong S[1]
[1]Alfred Hospital, Melbourne, Australia

Introduction: Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary cancer treatment for relapsed or refractory large B cell lymphoma1. Common toxicities of CAR-T therapy include cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) ranging from confusion and headache, to encephalopathy and seizures1. The immune effector cell-associated encephalopathy (ICE) score is a standardized approach for ICANS monitoring, and requires the patient to write a sentence2. ICE score has many limitations, including utility in patients with visual or hearing impairment. Here, we report the first case of a legally blind patient treated with CAR-T therapy, where ICE assessment was modified to cater for his sensory needs.

Case Report: GM is a 73-year-old high functioning man with congenital retinal blindness. He relapsed after 2 lines of chemotherapy for non-germinal centre DLBCL, and proceeded to CAR-T cell therapy (Axicabtagene ciloleucel).

He was extensively discussed in our multidisciplinary team with neuro-immunology and neuro-psychology teams prior to CART infusion for alternative methods of ICANS monitoring. The ICE score (out of 10) was modified; the handwriting item was omitted and the visual naming task (naming objects in the room) was replaced with an auditory naming task (naming objects from a verbal description), with a total ICE score of 9. GM was reviewed daily as an inpatient by the neurology team, and comprehensive neurology and neuropsychology assessments were conducted. He developed Grade 1 CRS and Grade 1 ICANS (right hand sensory/motor deficit) on day 1. CRS was managed with a single dose Tocilizumab (IV 8mg/kg) and ICANS with dexamethasone IV 10mg QID (weaned over five days). At the time of writing, he is D21 post infusion, and his CRS and ICANS have resolved.

Conclusion: The modified ICE score was an effective method for monitoring ICANS, and can be used for patients with visual impairment.

References

1. Shah, N. N., & Fry, T. J. (2019). Mechanisms of resistance to CAR T cell therapy. *Nature reviews Clinical oncology*, *16*(6), 372-385.

2. Lee, D. W., Santomasso, B. D., Locke, F. L., Ghobadi, A., Turtle, C. J., Brudno, J. N., Maus, M. V., Park, J. H., Mead, E., Pavletic, S., Go, W. Y., Eldjerou, L., Gardner, R. A., Frey, N., Curran, K. J., Peggs, K., Pasquini, M., DiPersio, J. F., van den Brink, M. R. M., Komanduri, K. V., Grupp, S. A., Neelapu, S. S. (2019). Astct consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells.. *Biology of Blood & Marrow Transplantation*, , *25*(4), 625-638. https://dx.doi.org/10.1016/j.bbmt.2018.12.758

Validation of a targeted lymphoma NGS panel on a walk away automated NGS library preparation system, the Agilent Magnis NGS Prep system

Lin W[1], Belluoccio D[2], Bradshaw G[1], De Silva W[1], Chew E[1], Agarwal R[1]

[1]Austin Health, Heidelberg, Australia, [2]Agilent Technologies, Inc., Mulgrave, Australia

Aim: NGS helps to define diagnostic, prognostic & therapeutic targets in lymphoid malignancies. Currently, there are no commercial lymphoid NGS panels available on the market in comparison to myeloid NGS panels. Here we have designed a custom lymphoma NGS panel covering 37 genes of clinical importance. The assay has been validated for use with the Agilent Magnis NGS Prep System for automated NGS library preparation, reducing time-consuming and laborious manual handling by scientists.

Method: DNA isolated from blood, bone marrow or FFPE tissue was analysed using an Agilent SureSelect custom-designed lymphoma panel. The panel utilises the SureSelect XTHS2 chemistry, including molecular barcodes. Automated library preparation was performed on the Magnis. Library quantity and quality was assessed using High Sensitivity D1000 ScreenTape assay on the Agilent 4200 TapeStation system. Indexed and pooled libraries are sequenced using Illumina MiSeq (Reagent Kit V3 600 cycles). Sequencing data are processed using Alissa Reporter v1.2 and variant curation using Alissa Interpret v5.4.2.

Results: DNA samples with known variants, including commercially available SeraCare Seraseq NGS reference Lymphoma controls, were run as part of the initial validation of the panel. The expected limit of detection of the assay is aimed at 2% variant allele frequency (VAF) for SNVs and 5% for complex variants. The Magnis automates the 9-hour library preparation workflow, only requiring 15 minutes' set-up time. Magnis reagent kits are pre-plated with QR barcodes that are scanned by the instrument to ensure sample indexing and tracking is consistent.

Conclusion: Automated processes in the laboratory reduce manual hands-on workflows in the laboratory, allowing trained scientists to focus on tasks such as variant curation, patient reporting, assay development and validation, all whilst improving turnaround times for NGS assays. Use of targeted NGS panels in lymphoid malignancies are important tools to provide diagnostic, prognostic and therapeutic information for patient management.

Acquired angioedema associated with lymphoproliferative disorders

Ng J[1], Ooi M[2], Bennett S[1], Rady K[1], Choi P[1], Lee W[3], Cook M[3,4], Randall K[3], Pati N[1]
[1]Department of haematology, Canberra Hospital, Canberra, Australia, [2]Canberra Hospital, Canberra, Australia, [3]Department of immunology, Canberra Hospital, Canberra, Australia, [4]Centre for Personalised Immunology, John Curtin School of Medical Research, Australian National University, , Canberra, Australia

Aim: Acquired angioedema due to C1 esterase inhibitor deficiency (C1INH-AAE) is most associated with low-grade B-lymphoproliferative disorders (LPD). This diagnosis may be challenging due to the lack of awareness, and misdiagnosis of the angioedema symptoms as part of the hematologic disorder.

Method: We discuss 4 cases of C1INH-AAE associated with low-grade B-LPD including the diagnostic challenges and management.

Results: Four males (53-79 years) presented with angioedema affecting different organs without constitutional symptoms, lymphocytosis, cytopenia or lymphadenopathy. C1INH-AAE was diagnosed based on complement C4, C1INH level and function. Bone marrow examination (BME) confirmed various low-grade B-LPDs.

C1INH-AAE should be considered in patients presenting with isolated angioedema without urticaria. It is most associated with LPD (62.8%), followed by monoclonal gammopathy of uncertain significance (27.7%), autoimmune disorders (10.6%) and solid organ tumours (5.3%). The prevalence was 10.7% in a retrospective study screening 131 patients with various LPD using complement and C1INH level but only 2.3% experienced significant angioedema.

The laboratory diagnosis is suggested by reduced complement C4, C1q, C1INH level and function. However, these may fluctuate during the clinical course. The presence of autoantibodies to C1INH has been known since the 1980s, but without commercial autoantibody assays their presence can only be inferred.

Our cases illustrate that the underlying LPD may be subtle. Multimodal screening is recommended including physical exam, full blood count, lactate dehydrogenase, serum protein electrophoresis, peripheral blood immunophenotyping, and CT of chest, abdomen, and pelvis.

As our cases illustrate, treatment of the underlying B-LPD commonly leads to improvement or resolution of the AAE, both clinically and biochemically. Hence, C4, and C1INH levels and function are useful for monitoring disease progress.

Conclusion: Awareness of C1INH-AAE can lead to an early diagnosis of haematological malignancies. The absence of constitutional symptoms emphasizes the need for a comprehensive multimodal approach to screening for LPD in C1INH-AAE. C4, C1INH level and function are useful for monitoring disease activity.

Detection of double hit lymphoma from tissue and cell free DNA using next generation sequencing

Nguyen T[1], Bennett R[1], Phung V[1], Yap Y[1], Yerneni S[1], Thompson E[1,2], Blombery P[1,2]
[1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]University of Melbourne, Melbourne, Australia

Background/Aims: High grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (HGBL-DH) is an aggressive mature B cell lymphoma typically identified by FISH assays. We aimed to assess the performance of a next generation sequencing (NGS)-based assay to detect HGBL-DH. In addition, we aimed to assess the detection by cell free DNA (cfDNA).
Methods: Stored tissue and cfDNA samples from patients with HGBL-DH were identified. Targeted NGS was performed using a custom hybridisation-capture panel (DHL panel) targeting *MYC*, *BCL2*, *BCL6* and *IGH*, *IGK* and *IGL* loci. Samples were sequenced to mean 400x depth. Sequence variants, structural variants and copy numbers were assessed.

Results: DHL panel was applied to 16 tissues and 15 cfDNAs from 16 patients at varying clinical time points. NGS showed concordant rearrangements of *MYC*/*BCL2*/*BCL6* to FISH in 12/16 (75%) tissue samples. Of the identified *MYC* rearrangements, four involved *IGH*, two involved *IGL* and five involved non-*IG* partners (*ARID5B*, *ZCCHC7*, *BCAT1*, *MIR1297* and *HDDC2*). One case had a pseudo-double-hit *BCL6*-*MYC* translocation representing a sub-entity distinct from conventional HGBL-DH. Notably this patient remained in clinical remission three years after treatment. In one sample multiple *MYC* rearrangements were detected consistent with the presence of intratumoural heterogeneity.

On plasma testing, all expected *MYC*/*BCL2*/*BCL6* rearrangements were detectable in 5/15 cfDNA samples when the tumour fraction (computed using aberrant somatic hypermutation variants) was greater than 20%. Non-detection in cfDNA was observed in samples with <20% tumour fraction or suboptimal input. Serial monitoring of cfDNA in two patients showed progressive reduction of cfDNA abnormalities tracking with reducing tumour burden on PET.

Conclusion: Targeted NGS can feasibly detect HGBL-DH from tissue DNA. Although limited by pre-analytical variables, cfDNA may also be feasibly used to demonstrate the genetic aberrations present. Potential analytical benefits of NGS over FISH include resolution of gene partners and identification of “pseudo” HGBL-DH.

Distinguishing Richter Transformation from de novo Burkitt Lymphoma in Chronic Lymphocytic Lymphoma using a multimodality diagnostic pathway

Noor W[1], Zirek Y[1], Cupit D[1], Diano G[1], Hutchinson A[1], Harrup R[1], Nelson N[1]
[1]*Royal Hobart Hospital, Hobart, Australia*

Background: Chronic lymphocytic leukaemia (CLL) is an indolent mature B-cell neoplasm that can evolve to an aggressive phenotype with a biological switch known as Richter transformation (RT). Distinguishing RT from de novo Burkitt lymphoma (BL) has therapeutic and prognostic implications but requires genomic studies to establish clonal relationship. Access, cost and extended turnaround times, however, limit the utility of many genomic assays in an urgent clinical setting.

Method: We describe rapid clarification of clonal unrelatedness using multimodal diagnostic approaches with fluorescence *in situ* hybridisation (FISH), expedited CLL-directed cell culture (<1 week) and conventional cytogenetics.

Case Report: A 76-year-old male presented with 2 weeks' history of constitutional symptoms. He had lymphocytosis (33 x 10^9/L), thrombocytopenia (20 x 10^9/L) and biochemical spontaneous tumour lysis syndrome. There was no relevant past medical history.
Initial bone marrow morphology and flow cytometry was suspicious for concomitant CLL and de novo BL rather than RT with two distinct abnormal B-cell populations with κ and λ-light chain restriction respectively.
Urgent FISH was supportive of BL. Expedited karyotyping confirmed a clonally distinct CLL population with trisomy 12 and a separate clonal population with -Y and t(8;14)(q24;q32) indicative of BL without trisomy 12.
Patient was commenced on BL-directed therapy, R CHOP.

Conclusion: Expedited conventional cytogenetics and FISH enabled diagnostic certainty of the occurence of concomitant CLL and de novo BL rather than Richter's transformation, in a clinically meaningful time frame to guide therapeutic decision making.

An approach to the management of Plasmablastic lymphoma

Ranjan A[1], Dubey H[1], Tanwar P[1]
[1]*All India Institute of Medical Sciences, New Delhi, India*

Background: Plasmablastic Lymphoma (PBL) is an aggressive B- cell lymphoma, usually associated with immunocompromised conditions.

Method: A retrospective study

Result: Out of 11 patients, male and female were 7(63.6%) and 4 (36.3%) respectively with median age 40 years (IQR 20-48). Retrovirus positive cases were 3/11 (27.2%) patients, one of them being hepatitis B carrier also. Mass in the head and neck region was the most common presentation 6/11 (54.5%) patients followed by abdominal lump 4/11 (36.3%) cases. One patient presented with pain and swelling in left arm. B symptoms were seen in 4/11 (36.3%) patients. At presentation, hematological parameters were within normal range. Biochemical parameters like liver and kidney function tests were normal except serum LDH 355 IU/L (IQR 271-566, Ref range 105-333 IU/L). Bone marrow (BM) infiltration by plasma cells was seen in 4/11 (36.3%) of cases and all of them had mass in head and neck region at presentation. Histopathology of tumor tissue biopsy from all the cases showed infiltration by large atypical lymphoid cells with plasmacytoid morphology, positive for CD138, MUM1 and negative for CD20 (except in one case). The most common therapeutic regimen was VPD and Bortezomib. Based on poor clinical condition of the patients, 3/11 (27.2%) patients were given dose adjusted EPOCH, 2/11(18.18%) BFM 90 and 2/11(18.1%) R- CHOP protocol. Single patient was lost to follow up before starting treatment. Following VPD & Bortezomib, 2 patients relapsed and they were given ICE regimen. Only 1 patient had died in the duration of follow up, the median follow up being 5 months.

Conclusion: PBL is not uncommon in immunocompetent patients, that most commonly presents with oral cavity, GIT or skin involvement. Generalized lymphadenopathy is rare. There is no standardized treatment protocol. In HIV positive patients DA-EPOCH is the first line therapy. The median overall survival of PBL patients is 6-19 months.

Key words: Plasmablastic lymphoma, Clinicopathological correlation, bone marro involvement, therapeutic regimen

Efficacy of polatuzumab combinations in relapsed/refractory diffuse large B cell lymphoma (RRDLBCL) trial-ineligible patients: a study from the Australian and New Zealand Lymphoma and Related Diseases Registry

Shaw B[1,2], Chung E[2], Wellard C[2], Yoo E[3], Bennett R[4], Birks C[5], Johnston A[6], Cheah C[3], Hamad N[7], Simpson J[8], Barraclough A[9], Ku M[10], Viiala N[11], Ratnasingam S[12], Armytage T[13], Chong G[14], Lee D[15], Manos K[16], Keane C[17], Wallwork S[17], Opat S[1,2], Hawkes E[18]

[1]Monash Health, Clayton, Australia, [2]Monash University, Melbourne, Australia, [3]Sir Charles Gairdner Hospital, Perth, Australia, [4]Peter MacCallum Cancer Centre, Parkville, Australia, [5]Concord Hospital, Concord, Australia, [6]Royal Hobart Hospital, Hobart, Australia, [7]St Vincent's Hospital, Sydney, Australia, [8]Port Macquarie Base Hospital, Port Macquarie, Australia, [9]Fiona Stanley Hospital, Murdoch, Australia, [10]St Vincent's Hospital, Melbourne, Australia, [11]Liverpool Hospital, Liverpool, Australia, [12]University Hospital Geelong, Geelong, Australia, [13]Gosford Hospital, Gosford, Australia, [14]Grampians Health Services, Ballarat, Australia, [15]Eastern Health, Melbourne, Australia, [16]Flinders Medical Centre, Adelaide, Australia, [17]Princess Alexandra Hospital, Brisbane, Australia, [18]Olivia Newton John Cancer Research Institute at Austin Health, Melbourne, Australia

Aim: To describe outcomes of patients treated with compassionate access polatuzumab for RRDLBCL in Australia and to compare to eligibility criteria and patient outcomes for the original GO29365 study and other 'real world' studies.

Method: Retrospective study of RRDLBCL pts ≥18y receiving Pola+/- Bendamustine/Rituximab (BR) from the Australian Lymphoma Registry (LaRDR). We analysed: demographics, disease & prior therapy details, eligibility status for landmark GO29365 study, outcomes and toxicity. Descriptive statistics, Kaplan-Meier method and the Cox proportional hazard model were used for the analyses.

Results: 58 patients were identified between 2019–2022, median age 63.0y, 62% male; 86% had stage III-IV disease; 61% had R-IPI ≥3. 70% had ≥2 prior therapies (38% >3 prior lines) with most being chemotherapy with rituximab.

74% failed ≥1 GO29365 study eligibility criteria and 47% failed ≥2 criteria. Pola+BR was given in 59%, Pola+rituximab in 24%; and single-agent in 8%. 27% completed all 6 cycles. Reasons for cessation included progressive disease 52%; bridging to other therapy 10%; death 10%; toxicity 4%. 8 pts received 1-2 subsequent lines of therapy (2 received CAR-T therapy).

ORR was 46% (25% CR). Median follow up was 18.8m (95%CI 5-29.4). Median OS was 3.5m (95% CI 2.7-5.9m). Median PFS was 2.3m (95% CI 1.9-4.0m). No difference in OS or PFS was observed for eligible vs non-eligible pts and failure of any one eligibility criteria category did not impact outcome.

Conclusion: Response rates were similar to other real-world studies but were lower than the registration trial. The high proportion of patients ineligible for the landmark pola-BR study and limited access to subsequent therapy potentially explain inferior response and survival outcomes in our cohort. Although a modest sample size, outcomes of novel therapies in real-world patients are likely influenced by factors outside of those related to trial eligibility such as adverse disease biology and additional comorbidities.

Table 1: Eligibility criteria that failed to be met and outcomes according to eligibility.

Factor	N (%)	Pts with data (n)	PFS Hazard Ratio (95%CI) pvalue	OS Hazard Ratio (95%CI) pvalue
Failed eligibility for the for the original GO29365 trial	43 (74)	58	0.80 (0.38-1.67) p = 0.55	0.99 (0.47-2.08) p = 0.97
Number of failed eligibility criteria		58		
0	15 (26)		N/A	N/A
1	16 (28)		1.35 (0.57-3.16) p = 0.50	1.72 (0.72-4.89) p = 0.22
2	16 (28)		0.77 (0.32-1.85) p = 0.56	0.83 (0.34-2.05) p = 0.69
3 or more	11 (19)		0.50 (0.20-1.28) p = 0.15	0.67 (0.26-1.77) 0.42
Number of significant co-morbidities*		58		
0	39 (67)		N/A	N/A
1	11 (19)		1.26 (0.60-2.62)p = 0.54	1.15 (0.53-2.51) p = 0.72
≥2	8 (14)		0.61 (0.25-1.48) p = 0.28	0.63 (0.24-1.66) p = 0.36
Treatment related failed eligibility			0.73 (0.38-1.40) p = 0.34	0.75 (0.38-1.48) p = 0.40
Treatment with CART cell therapy within 100 days prior to commencing Polatuzumab	7 (13)	53	1.96 (0.81-4.70) p = 0.13	2.04(0.78-5.38) p = 0.15
Autologous transplant eligible	15 (33)	46	0.51 (0.25-1.03) p = 0.061	0.47 (0.22-1.02) p = 0.056
Disease-related failed eligibility			0.50 (0.26-0.99) p = 0.048	0.51 (0.24-1.06) p = 0.072
Transformed from indolent ineligible	13 (26)	51	0.51 (0.24-1.09) p = 0.083	0.49 (0.21-1.13) p = 0.095
Presence of CNS involvement	5 (10)	52	1.18(0.40-3.17) p = 0.83	1.18 (0.36-3.85) p = 0.79
Failed Organ function eligibility			1.11 (0.56-2.19) p = 0.76	0.99 (0.49-2.01) p = 0.97

Encouraging complete responses (CRs) with CDK9 inhibitor AZD4573 in Patients (pts) with Relapsed/Refractory (r/r) Peripheral T-cell Lymphoma (PTCL): Early trial analysis

Zinzani P[1], Feldman T[2], Collins G[3], Zain J[4], Khot A[5], Kim J[6], Morschhauser F[7], Kim T[8], Roderick J[9], Yoon J[9], Sharma S[10], Saeh J[9], Dai J[9], Olsson R[11], **Shortt J[12]**

[1]University of Bologna Institute of Hematology, Bologna, Italy, [2]Hackensack University Medical Center, Hackensack, USA, [3]Oxford University Hospitals NHS Foundation Trust, Oxford, UK, [4]City of Hope, Duarte, USA, [5]Peter MacCallum Cancer Centre, , Australia, [6]Yonsei University, Seoul, South Korea, [7]CHRU Lille, Hopital Claude Huriez, Lille, France, [8]Seoul University National Hospital, Seoul, South Korea, [9]AstraZeneca, Boston, USA, [10]AstraZeneca, South San Francisco, USA, [11]AstraZeneca, Gothenburg, Sweden, [12]Monash University & Monash Health, Melbourne, Australia

Aim: Here we report the efficacy and safety of AZD4573 monotherapy in a phase 2a study of pts with r/r PTCL (NCT05140382).

Methods: Pts in this single-arm, open-label study were ≥18 years old, had ECOG PS ≤2, and ≥1 prior line of therapy including an alkylating agent and/or anthracycline. Primary cutaneous and leukemic PTCL subtypes were excluded. Each pt received an intra-pt ramp-up: 6 mg on day 1, 9 mg on day 8, then 12 mg on day 15, continuing QW thereafter. The primary objective was efficacy by investigator-assessed ORR (Lugano 2014 criteria); secondary objectives included efficacy by complete response (CR) rate, duration of response, progression-free survival and overall survival; safety and tolerability; and pharmacokinetics (PK).

Results: Eighteen pts received AZD4573; median age was 63.0 years, 66.7% were male and median number of prior regimens was 3.0. By 1 Feb 2023, efficacy was evaluable in 12 pts who had received at least one 12 mg dose. The ORR was 3/12 (25.0%, all CRs) in the efficacy-evaluable set. The CRs lasted 7.7 wks to 17.4+ wks. An additional complete metabolic response was observed in a pt after initial progressive disease. Safety was evaluable in 18 pts who received ≥1 dose. Treatment-emergent adverse events (TEAEs) occurred in 16 pts (88.9%), all of which were Grade ≥3. Key Grade ≥3 TEAEs were neutropenia (55.6%) and increased AST (22.2%). Two pts (11.2%) discontinued due to TEAEs (hospitalisation and septic shock, n=1 each). Serious TEAEs were reported in 72.2% and were deemed treatment-related by investigators in 61.1%. Grade 5 treatment-related AEs were reported in 2 pts (11.1%, both septic shock).

Conclusions: Preliminary results show encouraging clinical activity with AZD4573 monotherapy in pts with r/r PTCL, including 3 CRs and one CMR after initial PD. Safety and PK profiles are consistent with the phase 1 study with no unexpected findings, and the study continues to expand in the PTCL population. EA - previously submitted to EHA 2023.

Health-related quality of life (HRQOL) in patients with Waldenström macroglobulinemia (WM) treated with zanubrutinib or ibrutinib: results from long-term follow-up of the phase 3 ASPEN trial

Tam C[1], Tedeschi A[2], Owen R[3], Buske C[4], Leblond V[5], Dimopoulos M[6], Garcia-Sanz R[7], Castillo J[8], Trotman J[9], Treon S[8], Yang K[10], Tang B[10], Allewelt H[10], Patel S[10], Chan W[10], Cohen A[10], Schneider J[10], Barnes G[10]

[1]Alfred Health and Monash University, Melbourne, Australia, [2]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [3]St James University Hospital, Leeds, UK, [4]Institute of Experimental Cancer Research, CCCU, University Hospital Ulm, Baden-Württemberg, Germany, [5]Sorbonne University, Pitié Salpêtrière Hospital, Paris, France, [6]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, Athens, Greece, [7]Hospital Universitario de Salamanca, Salamanca, Spain, [8]Dana-Farber Cancer Institute, Boston, USA, [9]Concord Repatriation General Hospital, Sydney, NSW, Australia, [10]BeiGene USA, Inc, San Mateo, USA

Aim: HRQOL outcomes were evaluated in patients with WM who received zanubrutinib, a next-generation Bruton tyrosine kinase inhibitor, or ibrutinib in the randomized, open-label, phase 3 ASPEN (NCT03053440) study. Data from cohort 1 (*MYD88* mutations) in the intention-to-treat (ITT) population and in patients who achieved complete response (CR), or very good partial response (VGPR) are reported.

Method: Patient-reported outcomes (PROs) were assessed as exploratory endpoints via EORTC QLQ-C30 and EQ-5D-5L VAS scores. Patients completed questionnaires at baseline (cycle 1 day 1), every 3 cycles up to cycle 13, and then every 6 cycles (28-day cycles). Differences in PRO endpoints of global health status, physical and role functioning, and symptoms of fatigue, diarrhea, and nausea/vomiting were assessed between arms.

Results: Cohort 1 enrolled 201 patients (zanubrutinib, n=102; ibrutinib, n=99). Adverse events leading to dose holds or reductions, drug discontinuation, or death were higher with ibrutinib vs zanubrutinib. Adherence rates were high (zanubrutinib, 92%-97%; ibrutinib, 89%-98%). In the ITT population, diarrhea and nausea/vomiting scores were stable from baseline through all key clinical cycles with zanubrutinib; patients receiving ibrutinib had worsening of diarrhea and nausea/vomiting from baseline. In other key PRO endpoints, improvements from baseline were observed with both treatments but were not significantly different (**Table**). Median time to VGPR was shorter with zanubrutinib vs ibrutinib (8 vs 17 mo; CR+VGPR response rate, 38.2% vs 25.3%; *P*=.0374). Patients who achieved VGPR by cycle 25 with zanubrutinib (n=31) had generally better PRO endpoint outcomes than those receiving ibrutinib (n=17). Among patients achieving VGPR, differences between arms were clinically meaningful at cycles 7 and 25 for physical functioning and fatigue. Outcomes were worse with ibrutinib vs zanubrutinib in cycle 4 for diarrhea and nausea/vomiting.

Conclusions: Zanubrutinib was associated with greater improvements in HRQOL vs ibrutinib in patients with WM and *MYD88* mutations in ASPEN.

Table. Treatment Difference in Key PRO Endpoints (ITT Population) at Key Clinical Cycles[a]

PRO	Treatment difference between zanubrutinib and ibrutinib arms (95% CI)			
	Cycle 4	Cycle 7	Cycle 13	Cycle 25
GHS/QOL	−2.35 (−8.53 to 3.84)	−0.65 (−6.10 to 4.80)	−2.37 (−7.58 to 2.84)	−1.07 (−7.11 to 4.97)
Physical functioning	−0.18 (−5.37 to 5.00)	1.76 (−3.59 to 7.11)	−2.80 (−8.09 to 2.48)	0.53 (−4.23 to 5.29)
Role functioning	−2.85 (−10.36 to 4.67)	−1.81 (−9.27 to 5.65)	1.53 (−5.80 to 8.86)	3.02 (−3.73 to 9.83)
Diarrhea	**−7.26 (−12.62 to −1.90)[b]**	−4.90 (−10.63 to 0.84)[c]	−3.37 (−8.67 to 1.93)	0.57 (−4.76 to 5.91)
Fatigue	−1.76 (−8.14 to 4.62)	0.34 (−5.52 to 6.20)	1.10 (−4.81 to 7.01)	−0.05 (−6.34 to 6.24)
Nausea/vomiting	**−5.57 (−9.49 to −1.66)[d]**	0.80 (−1.62 to 3.21)	−1.52 (−3.85 to 0.81)	−0.33 (−3.13 to 2.47)

Descriptive analysis was performed using all scales. Differences between arms were assessed with a linear mixed-effects model for repeated measures. The model includes repeated measurements of the PRO endpoints up to cycle 25 as the dependent variable and the baseline score and treatment arm by timepoint interaction as covariates. An unstructured covariance matrix was used. Clinically meaningful differences (defined as a ≥5 point difference from baseline) are in bold.
GHS, global health status; ITT, intention to treat; PRO, patient-reported outcome; QOL, quality of life.

[a] Key clinical cycles corresponding to the median time to major response; [b] P=.008; [c] P=.093; [d] P=.0055.

Comparison of bleeding-related events in patients who received pirtobrutinib with and without antithrombotic agents

Tam C[1,2], Lamanna N[3], Jurczak W[4], Kontos A[5], Konig H[5], Ruppert A[6], Tsai D[5]
[1]Alfred Hospital, Melbourne, Australia, [2]Monash University, Melbourne, Australia, [3]Herbert Irving Comprehensive Cancer Center, Columbia University, , USA, [4]Maria Sklodowska-Curie National Research Institute of Oncology , , Poland, [5]Loxo@Lilly, Indianapolis, USA, [6]Eli Lilly and Company, Indianapolis, USA

Aim: Pirtobrutinib, a non-covalent (reversible) bruton tyrosine kinase inhibitor (BTKi) FDA-approved for treatment of R/R MCL, demonstrated efficacy and tolerability across B-cell malignancies. Bleeding events (BEs) in patients treated with pirtobrutinib and concomitant antithrombotic therapy (CAT) have not been reported.

Method: Patients with B-cell malignancies (317 CLL, 166 MCL, 290 other) enrolled in open-label Phase 1/2 BRUIN, who received pirtobrutinib with CAT were analyzed for BEs. CAT (direct-factor XA inhibitors, heparin anticoagulants, platelet aggregation inhibitors) at time of enrolment was permitted (excluding warfarin). Pirtobrutinib was administered QD (28-day cycles) until disease progression/discontinuation due to toxicity. CTCAE V5.0 determined grade/type of BEs.

Results: 773 patients (29July2022) received ≥1 pirtobrutinib dose; 216 with CAT (median age: 72years [IQR 65-77]). Median time on pirtobrutinib with and without CAT: 10.6 (IQR 4.0-19.9) and 9.3months (IQR 3.1-17.3). Any-grade BEs were reported in 44.9% patients with CAT vs 32.5% without (Table). >90% were grade≤2. Most common BEs in patients with CAT were contusion (22.7%), hematuria (5.6%), epistaxis (5.1%), petechiae (3.7%), hematoma (3.2%). Of those using CAT, 2 (0.9%) grade3 BEs were deemed pirtobrutinib related: upper GI bleeding with anemia and hemarthrosis from a knee injury). Eleven patients (2%) without CAT had Grade≥3 BEs. Hemorrhage/hematoma occurred in 13/79 (16.5%), 10/39 (25.6%), and 18/112 (16.1%) patients who received direct factor XA inhibitors, heparins, and platelet aggregation inhibitors (some >1 class), respectively. Among patients using CAT, median time to onset of BE was 8.1weeks; median BE duration was 2.1weeks (Table). Among patients using CAT, BEs required dose interruption of pirtobrutinib in 5 patients; no BEs led to dose reduction/permanent discontinuation of pirtobrutinib.

Conclusion: While CAT with pirtobrutinib was associated with a slightly increased rate of BEs vs pirtobrutinib alone; most events were grade≤2. High-grade BEs were infrequent (<2%). This supports safety of pirtobrutinib in patients requiring CAT.

Table: Summary of bleeding events in patients who received pirtobrutinib with or without antithrombotic medication.

	Pirtobrutinib Monotherapy N=773			
	Concomitant Antithrombotic N=216		No Concomitant Antithrombotic N=557	
Summary of Bleeding Events	**All Grades n (%)**	**Grade ≥3[e] n (%)**	**All Grades n (%)**	**Grade ≥3 n (%)**
Bleeding[a,b]	97 (44.9)	6 (2.8)	181 (32.5)	11 (2.0)
Bruising[c]	60 (27.8)	0 (0.0)	123 (22.1)	0 (0.0)
Hemorrhage/hematoma[d]	34 (15.7)	4 (1.9)	54 (9.7)	10 (1.8)
Hematuria	12 (5.6)	0 (0.0)	15 (2.7)	0 (0.0)
Gingival bleeding	3 (1.4)	0 (0.0)	2 (0.4)	0 (0.0)
Hemoptysis	3 (1.4)	0 (0.0)	1 (0.2)	0 (0.0)
Median Time to First Onset, Weeks (IQR)	8.1 (2.6-24)		4.1 (1.3-16.1)	
Median Duration, Weeks (IQR)	2.1 (0.6-4.3)		4.0 (1.1-7.9)	
Bleeding Events Requiring Dose Reduction or Discontinuation	0 (0.0%)		1 (0.2%)	
Bleeding Events Requiring or Prolonging Hospitalization	5 (2.3%)		9 (1.6%)	

IQR = interquartile range. [a]Patients may appear in more than one subcategory. Events occurring in ≥1% of patients are presented. [b]Adverse events shown are those that were previously associated with covalent BTK inhibitors. [c]Aggregate of contusion, petechia, ecchymosis, and increased tendency to bruise. [d]Aggregate of all preferred terms including hemorrhage or hematoma. [e]No grade 4-5 bleeding events occurred in patients with concomitant antithrombotic therapy.

Comparing the international prognostic indices R-IPI and IPI for diffuse large B-cell lymphoma in an Australian centre

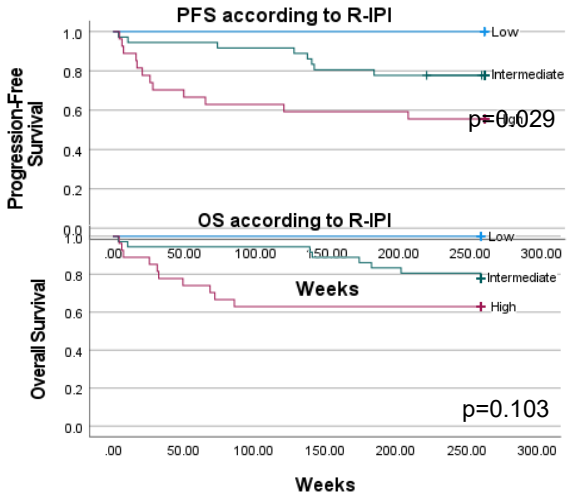
White E[1], Roncolato F, Shuttleworth C
[1]*St George Hospital, Kogarah, Australia*

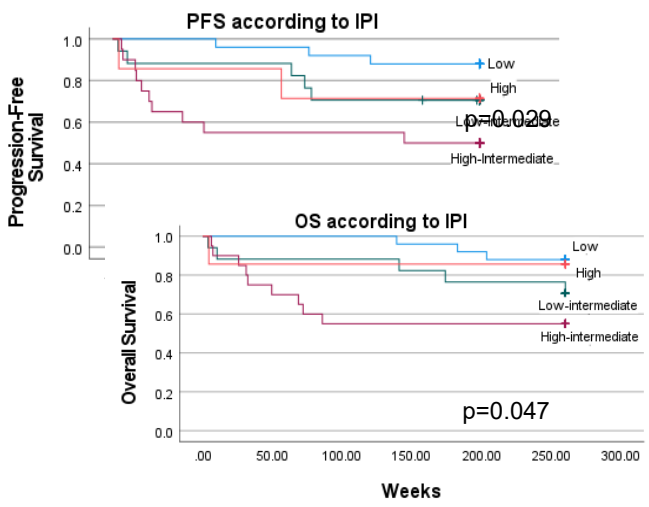
Aim: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma in Australia, carrying significant inter-patient variability in long term outcomes. A number of prognostic scores have been internationally validated for risk stratification including the International Prognostic Index (IPI) and its revised counterpart (R-IPI), with previous research suggesting superior utility of R-IPI. This study was performed to compare these indices in an Australia-specific context.

Method: We performed a retrospective cohort study via medical record review of patients newly diagnosed with DLBCL from 2013-15 in a Sydney hospital network, comparing the IPI and R-IPI in predicting progression-free and overall survival over a 4-year period. A total of 69 patients were included, and their outcomes examined using Kaplan-Meier analysis, with comparison between the groups using the log rank test.

Result: The median age was 69.0 years (27-91), with an overall 4-year PFS of 71.0%, and 4-year OS of 73.9%. Both the IPI and R-IPI were equally predictive of PFS (p=0.029 for both), while only IPI remained significantly predictive of OS (p=0.047). Clear curve separation between groups was maintained throughout the follow up period for all but the high-risk group in IPI, possibly secondary to low group number, although statistical significance with IPI was reached despite this.

Conclusion: Our retrospective study reaffirms, in an Australian setting, the validity of both the IPI and R-IPI scores in predicting PFS for patients with newly diagnosed DLBCL, although only IPI significantly predicted OS. The favourable outcomes demonstrated for even ‘high risk’ patients with both tools supports the increasing role of FISH in further stratifying risk.





Zanubrutinib plus obinutuzumab vs obinutuzumab in patients with relapsed/refractory follicular lymphoma: updated analysis of the Rosewood study

Yuen S[1], Trotman J[2], Ganly P[3], Zinzani P[4], Mayer J[5], Bijou F[6], de Oliveira A[7], Song Y[8], Auer R[9], Kim P[10], Huang S[11], Delarue R[12], Flowers C[13]

[1]Calvary Mater Newcastle, Waratah, Australia, [2]Concord Repatriation General Hospital, University of Sydney, Concord, Australia, [3]Department of Haematology, Christchurch Hospital, Christchurch, New Zealand, [4]Institute of Hematology "Seràgnoli", University of Bologna, Bologna, Italy, [5]Department of Internal Medicine-Hematology and Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic, [6]Institut Bergonié, Bordeaux, France, [7]Institut Català d'Oncologia (ICO) - Hospital Duran i Reynals, Barcelona, Spain, [8]Peking University Cancer Hospital and Institute, Beijing, China, [9]St Bartholomew's Hospital, Barts Health NHS Trust, London, UK, [10]BeiGene USA, Inc, San Mateo, USA, [11]BeiGene (Shanghai) Co, Ltd, Shanghai, China, [12]BeiGene International GmbH, Basel, Switzerland, [13]Department of Lymphoma/Myeloma, The University of Texas MD Anderson Cancer Center,, Houston, USA

Aim: In an early-phase study, the combination of zanubrutinib plus obinutuzumab (ZO) was well tolerated and showed a signal of efficacy in patients with follicular lymphoma (FL) (Tam. *Blood Adv.* 2020). ROSEWOOD (NCT03332017) is a phase 2 study designed to assess the efficacy and safety of ZO vs obinutuzumab (O) in patients with relapsed/refractory (R/R) FL. An updated analysis with a median follow-up of 20.2 months is reported.

Method: Patients (N=217) with R/R FL (grade 1-3a) who received ≥2 lines of therapy including an anti-CD20 antibody and alkylating agent were randomized 2:1 to receive ZO (n=145) or O (n=72). Zanubrutinib was given at 160 mg twice daily until progression or unacceptable toxicity. The primary endpoint was overall response rate (ORR) by independent central review. Other endpoints included duration of response (DOR), progression-free survival (PFS), overall survival, and safety. Time to next treatment was also assessed.

Result: In the study population (median age, 64 years; median prior lines, 3 [range, 2-11]), 52.5% of patients were refractory to rituximab; 98.6% received prior immunochemotherapy. ORR was 69.0% (ZO) vs 45.8% (O). DOR at 18 months was 69.3% (ZO) vs 41.9% (O). Median PFS was 28.0 months (ZO) vs 10.4 months (O) (hazard ratio, 0.50; 95% CI, 0.33-0.75; *P*=.0007). Additional efficacy results are in the **Table**. Nonhematologic treatment-emergent adverse events (any grade) that occurred more frequently with ZO vs O (>5% difference) were petechiae and herpes zoster infection (6.3% vs 0% for both); in patients receiving O, pyrexia (13.3% vs 19.7%) and infusion-related reactions (2.8% vs 9.9%) occurred more frequently. Exposure-adjusted incidences of infection and cytopenia were similar; incidence of hemorrhage (all grade) was 2.4 (ZO) vs 1.3 (O) persons/100 person-months.

Conclusion: ZO demonstrated meaningful efficacy and a manageable safety profile in patients with heavily pretreated R/R FL and represents a potential novel therapy.

Table. Efficacy Results

	ZO	O	HR (95% CI)	2-sided *P* value
Primary endpoint				
ORR by ICR, %	69.0	45.8	-	.0012
Other endpoints				
Complete response rate by ICR, %[a]	39.3	19.4	-	.0035
18-month DOR rate by ICR, %[a]	69.3	41.9	-	-
PFS by ICR, median, months[a]	28.0	10.4	0.50 (0.33-0.75)	.0007
TTNT, median, months	NE	12.2	0.34 (0.22-0.52)	<.0001
OS rate at 24 months, %[a]	77.3	71.4	-	-
OS, median, months[a]	NE	34.6	0.62 (0.35-1.07)	.0845

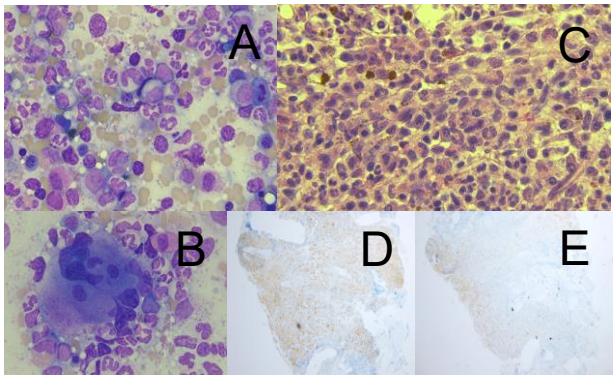
DOR, duration of response; HR, hazard ratio; ICR, independent central review; NE, not estimable; O, obinutuzumab; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TTNT, time to next treatment; ZO, zanubrutinib plus obinutuzumab.
[a] Secondary endpoint.

Concurrent staphylococcal scalded skin syndrome with subclinical pemphigus foliaceus in systemic mastocytosis with myelodysplastic/myeloproliferative neoplasm

Lee V[1,3], Kim J[2,8], Kitson J[3], Fernandez Penas P[4,5,6,7,8], Fuller S[3,8], Blyth E[1,5,8,9]

[1]Department of Haematology, Westmead Hospital, Westmead, Australia, [2]Department of Tissue Pathology & Diagnostic Oncology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, NSW Health Pathology, Westmead, Australia, [3]Department of Haematology, Nepean Hospital, Kingswood, Australia, [4]Department of Dermatology, Westmead Hospital, Westmead Hospital, Australia, [5]The Westmead Institute for Medical Research, Westmead, Australia, [6]ACRF Australian Centre of Excellence in Melanoma Imaging and Diagnosis, Sydney, Australia, [7]Westmead Applied Research Centre, University of Sydney, Westmead, Australia, [8]Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Sydney, Australia, [9]Sydney Cellular Therapies Laboratory, Sydney, Australia

Figure 1 – Trephine imprints with dysplastic neutrophils and mast cell infiltrates (A); dysmorphic megakaryocyte (B). (C) Spindle shaped mast cells on trephine. Mast cells visualised with (D) CD25 and (E) CD2 stains.



Introduction: We describe a novel case of concurrent staphylococcal scalded skin syndrome (SSSS) with subclinical pemphigus foliaceus (PF) in concurrent systemic mastocytosis (SM) and myelodysplastic/myeloproliferative neoplasm (MDS/MPN). Only one case of SM with pemphigus has been previously reported.

Case: A 46-year-old female with mast cell infiltrate on gastroscopy and raised serum tryptase was referred for haematological evaluation. Blood films featured left shifted granulocytes and thrombocytosis with megakaryocyte fragments. Bone marrow biopsy demonstrated hyperplasia of granulocytes and atypical megakaryocytes, morphologically suggesting MPN. CD117 positive mast cells were increased, co-expressing CD25 without CD2. C-Kit D816V was positive, overall leading to diagnosis of SM with associated haematological neoplasm.

Mastocytosis persisted despite prednisone, hydroxyurea 500mg BD, and pegylated interferon (peg-IFN) 135mcg weekly. Midostaurin did not improve endoscopy findings. Cladribine was poorly tolerated and improvement in SM symptoms was short-lived. She recommenced hydroxyurea and peg-IFN, but developed lymphadenopathy and hepatosplenomegaly. Progress blood films were leukoerythroblastic with eosinophilia, dysplastic neutrophils, and marked thrombocytosis. Repeat bone marrow biopsy demonstrated dysplasia (figure 1A-B), with spindled mast cells (figure 1C) co-expressing CD25 and CD2 (figure 1D-E), suggesting SM with MDS/MPN. Lymph nodes biopsies confirmed nodal involvement.

Whilst preparing for allogeneic stem cell transplant (alloSCT), our patient developed painful flaccid bullae, without mucosal involvement involving 60% of the body (BSA). Microbiology and clinical appearance suggested SSSS. With antibiotic treatment, BSA involvement reduced to 15%. Skin biopsy demonstrated suprabasilar (figure 2A) and subcorneal blister with neutrophils and interface reaction but direct immunofluorescence was positive (figure 2B). Prednisone 1mg/kg was added to the treatment. DSG1 and envoplakin antibodies were detected by ELISA.

Conclusion: With the diagnosis of delayed SSSS complicated with subclinical
PF, she was treated with two doses of rituximab 1g. Current management is supportive until sufficient recovery is achieved to proceed with curative alloSCT.

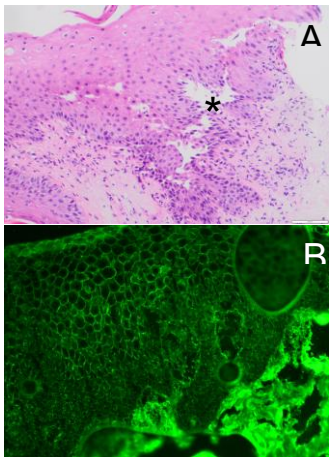


Figure 2 – (A) Suprabasilar blisters. (B) Direct immunofluorescence showing intercellular space deposition of IgG in the epidermis.

Bone Marrow Lymphocyte Subsets in Elderly Patients with Myelodysplastic Syndrome Treated with Azacitidine

Mahaliyana T[1], McNamara N[2], Nguyen T[2], Garcia R[2], Jayaratne N[2], Motum P[2,3,4], Getta B[3,4]
[1]Bankstown Hospital, Bankstown, Australia, [2]New South Wales Health Pathology, Liverpool, Australia, [3]Haematology Department, Liverpool Hospital, Liverpool, Australia, [4]University of New South Wales, Randwick, Australia

Aim: Myelodysplastic Syndromes (MDS) are malignant disorders of aging, with worse prognosis reported with increasing frailty[vi].

Immunosenescence in ageing is associated with a cytotoxic profile, with increased $CD8^+$ cytotoxic T-cell, decreased $CD4^+$ T-helper cell count and $CD4^+$:$CD8^+$ ratio[vii]. Multiparametric Flow Cytometry (MFC) represents a potential modality for adding to assessment of frailty and prognosis.

We report on pre-treatment flow cytometric characteristics of elderly patients aged ≥65 years with myelodysplastic syndromes treated with azacitidine and its prognostic significance.

Objectives:

- Comparison of T-lymphocyte subsets in elderly patients with MDS vs normal controls
- Median Overall Survival (OS) as a function of T-lymphocyte subset proportion in elderly patients treated with azacitidine

Method: 42 azacitidine-treated patients aged ≥65 years with MDS were identified from the SWSLHD myeloid database. Pre-treatment MFC was re-analysed for lymphocyte subsets. Statistical analysis was performed using SPSS.

7 patients undergoing bone marrow biopsy without evidence of myeloid disorder were analysed prospectively as normal controls, using MFC.

Results: Patients with MDS had increased $CD3^+/CD4^-$ T-lymphocyte count and reduced $CD4^+$:$CD4^-$ ratio compared with normal controls, in keeping with an immunosenescent, cytotoxic profile.

However, $CD3^+$ T-lymphocyte count, $CD3^+/CD4^-$, $CD3^+/CD4^+$ T-lymphocyte count or $CD4^+$:$CD4^-$ ratio were not associated with overall survival.

Conclusion: Elderly patients with MDS exhibit an immunosenescent, cytotoxic bone marrow phenotype. This phenotype was not associated with overall survival with azacitidine treatment, possibly due to inadequate numbers. Re-analysis of historical MFC was a feasible research methodology.

Flow cytometric detection of chromosome 17 abnormalities in circulating CD34-positive cells in Myelofibrosis

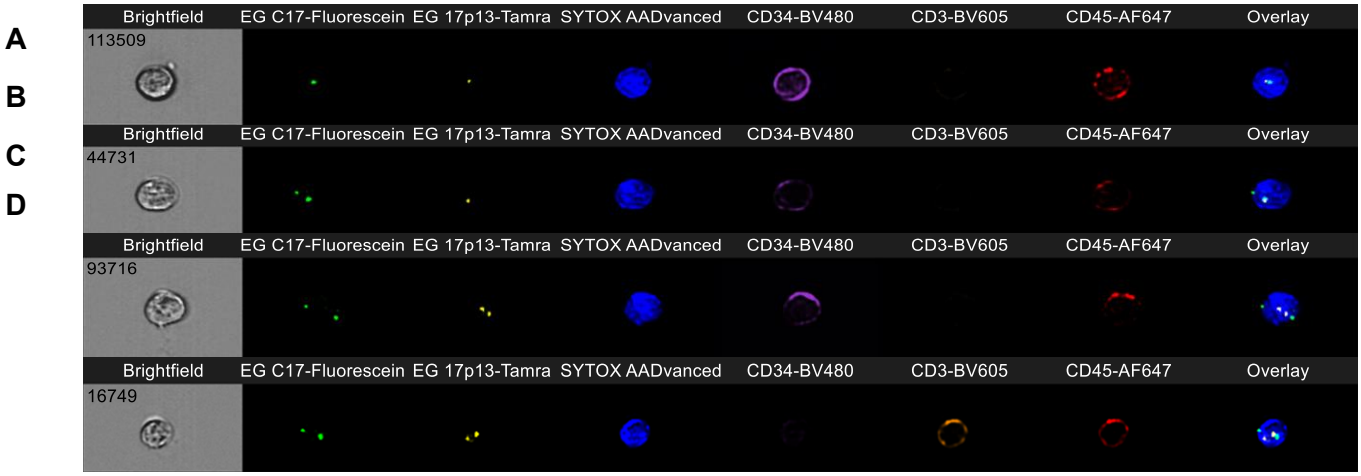
Ng Z[1,2], Collinson R[1], Hamilton R[1], Kruger P[3], Linden M[1], Fuller K[1], Guo B[1], Erber W[1,2]
[1]School of Biomedical Sciences, University of Western Australia, Crawley, Australia, [2]Department of Haematology, Royal Perth Hospital, Perth, Australia, [3]Department of Haematology, Fiona Stanley Hospital, Murdoch, Australia

Background: Cytogenetic analysis, typically performed on bone marrow, is integral for the management of myeloproliferative neoplasms. Marrow fibrosis often precludes this. We aimed to determine whether FISH on circulating CD34-positive cells would provide an alternative approach. We assessed this using an imaging flow cytometric method that can assess chromosomes in cells of interest identified by their immunophenotype ("immuno-flowFISH").

Methods: Mononuclear cells were isolated using density gradient centrifugation from EDTA-anticoagulated blood from a 76-year-old with myelofibrosis (leucocyte count 4.14 $x10^9$/L). The cells were incubated with fluorescently conjugated monoclonal antibodies: CD34-BV480, CD45-AF647 and CD3-BV480. After fixation and permeabilisation, DNA was denatured and hybridised with FISH probes for the chromosome 17 centromere (C17-Fluorescein) and 17p13 locus (17p13-TAMRA). Following washing and nuclear counterstaining, 120,000 cells were acquired on the Amnis ImageStream®X Mark II imaging flow cytometer and data analysed using the IDEAS software.

Results: 11.8% of mononuclear cells were CD34/CD45-positive (equating to 0.45x10^9/L). Within this subset, two abnormal CD34-positive sub-populations were identified with differing chromosome 17 configurations. 32% had one FISH signal for both the centromere of chromosome 17 and 17p locus, indicating monosomy 17 (absolute count 0.15x10^9/L) (Figure 1A). Another 16% had one FISH signal for 17p13, but two for the centromere indicating del(17p) in 0.06x10^9/L cells (Figure 1B). The remaining CD34-positive cells and the CD3/CD45-positive control T-lymphocytes had dual spots for both probes (Figure 1C, 1D, respectively).

Figure 1



Conclusion: Immuno-flowFISH of this blood sample showed monosomy 17 and del(17p) in circulating CD34-positive cells at levels of 0.15 and 0.06x10^9/L (respectively), or 5% of the leucocyte differential count. The high sensitivity and specificity were achieved through acquisition of 120,000 cells, and analysing those with the immunophenotype of interest. This blood-based flow cytometric FISH method has potential as a monitoring tool for measurable low-level disease and an indicator of clonal evolution in myeloproliferative neoplasms.

Bone marrow fibrosis changes do not correlate with efficacy outcomes in myelofibrosis: analysis of more than 300 JAK inhibitor-naïve patients treated with momelotinib or ruxolitinib

Perkins A[1], Oh S[2], Verstovsek S[3], Gupta V[4], Platzbecker U[5], Gisslinger H[6], Devos T[7], Kiladjian J[8], McLornan D[9], Fox M[10], McMullin M[11], Mead A[12], Egyed M[13], Mayer J[14], Sacha T[15], Kawashima J[16], Huang M[16], Strouse B[16], Mesa R[17]

[1]Alfred Health,The Australian Centre For Blood Diseases,Monash University, Melbourne, Australia, [2]Washington University School of Medicine, St Louis, USA, [3]The University of Texas MD Anderson Cancer Center, Houston, USA, [4]Princess Margaret Cancer Centre,University of Toronto, Toronto, Canada, [5]University of Leipzig Medical Center, Leipzig, Germany, [6]Medical University of Vienna, Wien , Austria, [7]University Hospitals Leuven and Laboratory of Molecular Immunology (Rega Institute),KU Leuen, Leuven, Belgium, [8]Université de Paris,AP-HP, Hôpital Saint-Louis,Centre d'Investigations Cliniques, Paris, France, [9]Guy's and St Thomas' NHS Foundation Trust, London, UK, [10]Vall d'Hebron University Hospital, Barcelona, Spain, [11]Queen's University, Belfast, UK, [12]Radcliffe Department of Medicine,University of Oxford, Oxford, UK, [13]Somogy County Kaposi Mór General Hospital, Kaposvár, Hungary, [14]Masaryk University and University Hospital Brno, Brno, Czech Republic, [15]Jagiellonian University Hospital, Kraków, Poland, [16]Sierra Oncology, Inc., San Mateo, USA, [17]Wake Forest University School of Medicine, Winston Salem, USA

Aim: To investigate changes in bone marrow fibrosis (BMF) in JAK inhibitor (JAKi)-naïve patients from the international, double-blind, randomized, phase 3 study SIMPLIFY-1 (Funding: Gilead Sciences, Sierra Oncology, Inc., a GSK company, NCT01969838) of momelotinib (MMB) versus ruxolitinib (RUX).

Method: BMF biopsies were collected at baseline, at Week (W) 24 of randomized treatment with MMB or RUX, and at W96 during open-label treatment with MMB. Assessments: BMF grading (WHO G0–G3), MFSAF symptom scoring, spleen volume imaging, transfusion independence (TI) status, and hemoglobin levels; OS was estimated using Kaplan-Meier analysis.

Results: Of patients randomized to MMB (N=215) and RUX (N=217), 211 and 213 had baseline BMF assessments, respectively, and most (59%) were G3. W24 paired biopsies were available in 144/211 and 160/213 patients on MMB and RUX, respectively; the proportion with stable/improved BMF was similar between arms (≥1G improvement: 22% vs. 23%; stable/improved BMF: 85% vs. 81%). In the MMB arm, 87% of patients with ≥1G BMF improvement and 76% with stable/worsening BMF were W24 TI responders (TI-R); 44% of patients in the RUX arm with ≥1G improvement and 56% with stable/worsening BMF were W24 TI-R. Regardless of BMF status, hemoglobin levels increased on MMB and decreased on RUX. No associations were noted between BMF and spleen/symptom outcomes in both arms. No improvement in OS was observed based on ≥1G BMF improvement (MMB: HR=0.78, p=0.5203; RUX→MMB: HR=1.27, p=0.4789). Patients with worsening BMF grade trended to have improved OS versus those without change (MMB: HR=0.570; RUX: HR=0.597) or improvement (HR=0.818; HR=0.523), but was not statistically significant.

Conclusion: Among JAKi-naïve patients with MF who received MMB or RUX, BMF changes were not associated with anemia improvement, or spleen and symptom outcomes.

Encore abstract: Oh S, *et al*. *Blood*. 2022;140 (suppl 1):821-823 (https://doi.org/10.1182/blood-2022-160409).

Transfusion independence response as a potential surrogate for overall survival in JAKi-experienced patients with myelofibrosis from MOMENTUM

Perkins A[1], Verstovsek S[2], Oh S[3], Kiladjian J[4], Platzbecker U[5], Passamonti F[6], Gerds A[7], Vannucchi A[8], McLornan D[9], Gupta V[10], Grosicki S[11], Al-Ali K[12], Lavie D[13], Kuykendall A[14], Lee S[15], Iurlo A[16], Ocroteala L[17], Kawashima J[18], Donahue R[18], Strouse B[18], Mesa R[19]
[1]Alfred Health,The Australian Centre For Blood Diseases,Monash University, Melbourne, Australia, [2]The University of Texas MD Anderson Cancer Center, Houston, USA, [3]Washington University School of Medicine, St Louis, USA, [4]Université de Paris, AP-HP,Hô pital Saint-Louis,Centre d'Investigations Cliniques, Paris, France, [5]University of Leipzig Medical Center, Leipzig, Germany , [6]University of Insubria, Varese, Italy, [7]ClevelandClinic Taussig Cancer Centre, Cleveland, USA, [8]University of Florence, Firenze, Italy, [9]Guy's and St Thomas' NHS Foundation Trust, London, UK, [10]Princess MargaretCancer Centre,University of Toronto, Toronto , Canada, [11]Medical University of Silesia, Katowice, Poland, [12]University Hospital of Halle (Saale), Halle, Germany, [13]Hadassah University Medical Center, Jerusalem , Israel, [14]Moffitt Cancer Center, Tampa, USA, [15]Catholic Hematology Hospital,The Catholic University of Korea,St. Mary's Hospital, Seoul, South Korea, [16]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [17]Filantropia Hospital, Craiova, Romania, [18]Sierra Oncology, Inc., San Mateo, USA, [19]Wake Forest University School of Medicine, Winston Salem, USA

Aim: To further understand the impact of momelotinib (MMB) on transfusion independence response (TI-R), overall survival (OS) and transfusion burden in JAK inhibitor (JAKi)–experienced patients with myelofibrosis (MF) who received MMB versus danazol (DAN) from the international, phase 3 MOMENTUM study (Funding: Sierra Oncology, Inc., a GSK company; NCT04173494).

Method: After the 24-week randomization (2:1) period with MMB 200 mg daily (N=130) versus DAN 600 mg daily (N=65), patients could receive open-label MMB. Eligibility: primary or post-ET/PV MF; DIPSS high risk, Int-2, or Int-1 MF; total symptom score ≥10; hemoglobin <10 g/dL; prior JAKi; and palpable spleen ≥5 cm. Anemia benefit was evaluated by TI-R (defined as absence of transfusions and no hemoglobin measurements below 8 g/dL during the 12 weeks before Week 24 [W24]); survival was estimated using Kaplan-Meier analysis.

Results: Highly anemic patients were included in MMB and DAN arms, with mean hemoglobin 8.1 g/dL and 7.9 g/dL; hemoglobin <8 g/dL in 48% and 49%; transfusion dependence (TD) in 49% and 52% at baseline. W24 TI-R rates were 31% and 20% with MMB and DAN (non-inferiority p=0.0064). Over 24 weeks, 35% of MMB patients had no transfusions versus 17% with DAN (OR=2.7; p=0.0107); regardless of baseline transfusion status, mean cumulative RBC units was less with MMB than DAN. *Post hoc* interim OS analysis showed that patients who achieved W24 TI-R had prolonged OS versus non-TI-R (HR=0.15; p=0.0364).

Conclusion: Analyses from MOMENTUM presented here demonstrated that MMB increased the likelihood of TI-R and lower transfusion burden versus DAN, and provide further support of W24 TI-R as a potential surrogate for improved OS.

Encore abstract: Verstovsek S, *et al*. *Blood*. 2022;140 (suppl 1): 6803-6805 (https://doi.org/10.1182/blood-2022-162877).

Impact of transfusion burden on health-related quality of life and functioning in patients with myelofibrosis: post hoc analysis of SIMPLIFY-1 and -2

Perkins A[1], Mesa R[2], Palandri F[3], Verstovsek S[4], Masarova L[4], Harrison C[5], Mazerolle F[6], Gorsh B[7], M'hari M[6], Wang Z[7], Ellis C[7], Deheshi S[8], Kawashima J[8], von Maltzahn R[9], Regnault A[6]
[1]Alfred Health,The Australian Centre For Blood Diseases,Monash University, Melbourne, Australia, [2]Wake Forest University School of Medicine, Winston Salem, USA, [3]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [4]The University of Texas MD Anderson Cancer Center, Houston, USA, [5]Guy's and St Thomas' NHS Foundation Trust, London, UK, [6]Modus Outcomes, Lyon, France, [7]GSK plc, Philadelphia, USA, [8]Sierra Oncology, Inc., San Mateo, USA, [9]GSK plc, London, UK

Aim: This exploratory post hoc analysis of the international, phase 3 SIMPLIFY-1 (NCT01969838) and SIMPLIFY-2 (NCT02101268) trials of momelotinib (MMB) in patients with myelofibrosis (MF) aimed to characterize the relationship between transfusion burden and patient-reported outcomes (PROs) (Funding: Gilead Sciences, Sierra Oncology, Inc., a GSK company).

Method: The pooled analysis set comprised both arms of the intent-to-treat populations of SIMPLIFY-1 (JAK inhibitor–naïve, MMB vs ruxolitinib [RUX]; N=432) and SIMPLIFY-2 (JAK inhibitor–exposed, MMB vs best available therapy [88% RUX]; N=156). Transfusion independent (TI): no transfusions and hemoglobin ≥8 g/dL in 12 weeks; transfusion dependent (TD): ≥4 units of transfusions or hemoglobin <8 g/dL in 8 weeks; transfusion requiring (TR): neither TI/TD. PRO assessments: SF-36v2 (physical functioning, role-physical, general health, vitality, social functioning, and mental health domains; norm-based scores [NBS]).

Results: Of the pooled analysis set (N=588), 503 had baseline SF-36v2 NBS; mean NBS in all domains were lower than the general population per the SF-36v2 Manual (50; SD, 10): physical functioning, 39.6; role-physical, 39.0; general health, 39.8; vitality, 43.6; social functioning, 43.2; mental health, 46.3. Mean NBS in all domains at baseline and week 24 (W24) were lower in TD than TI patients. At W24, among baseline TD patients (n=150), 75 remained TD, 40 became TI, 21 became TR, and 14 without data. TD patients who became TI experienced greater improvement in most domains versus patients who remained TD.

Conclusion: In SIMPLIFY-1 and SIMPLIFY-2, MF patients with TD had lower functioning and HRQOL than TI patients. TD patients who became TI at W24 had greater improvement across most domains than those who remained TD. These analyses highlight TD as a key patient-centered outcome.

Clinical outcomes of myelofibrosis patients following immediate transition to momelotinib from ruxolitinib

Perkins A[1], Mesa R[2], Verstovsek S[3], Platzbecker U[4], Gupta V[5], Lavie D[6], Giraldo P[7], Kiladjian J[8], Oh S[9], Devos T[10], Passamonti F[11], Vannucchi A[12], Pluta A[13], McLornan D[14], Kawashima J[15], Huang M[15], Strouse B[15], Harrison C[14]

[1]Alfred Health,The Australian Centre For Blood Diseases,Monash University, Melbourne, Australia, [2]Wake Forest University School of Medicine, Winston Salem, USA, [3]The University of Texas MD Anderson Cancer Center, Houston, USA, [4]University of Leipzig Medical Center, Leipzig, Germany, [5]Princess Margaret Cancer Centre,University of Toronto, Toronto, Canada, [6]Hadassah University Medical Center, Jerusalem, Israel, [7]Miguel Servet University Hospital and Centro de Investigacion Biomedica en Red de Enfermadades Raras (CIBERER), Zaragoza, Spain, [8]Université de Paris, AP-HP, Hôpital Saint-Louis,Centre d'Investigations Cliniques, Paris, France, [9]Washington University School of Medicine, St Louis, USA, [10]University Hospitals Leuven and Laboratory of Molecular Immunology (Rega Institute),KU Leuven, Leuven, Belgium, [11]University of Insubria, Varese, Italy, [12]University of Florence, Firenze, Italy, [13]Szpital Specjalistyczny w Brzozowie Podkarpacki Ośrodek Onkologiczny im. Ks. B. Markiewicza, Brzozów, Poland, [14]Guy's and St Thomas' NHS Foundation Trust, London, UK, [15]Sierra Oncology, Inc., San Mateo, USA

Aim: This retrospective analysis aimed to investigate the timing and clinical experience of direct transition from ruxolitinib (RUX) to momelotinib (MMB) among patients with myelofibrosis (MF).

Method: In the international, phase 3 SIMPLIFY-1 study (Funding: Sierra Oncology, Inc., a GSK company, NCT01969838), JAK inhibitor–naïve patients with MF (n=432) were randomized 1:1 to MMB or RUX. In SIMPLIFY-2 (NCT02101268), RUX-treated patients with MF (n=156) were randomized 2:1 to MMB or best available therapy (BAT). Following the 24-week randomized treatment period, patients could continue MMB (MMB→MMB), while those on RUX/BAT crossed over to open-label MMB (RUX/BAT→MMB) immediately without tapering/washout.

Results: At Week (W) 25 in SIMPLIFY-1, 197 patients transitioned from RUX→MMB 200 mg daily and 171 remained MMB→MMB, 81% of RUX group received MMB for 12 weeks following crossover. The first post-crossover assessment showed a rapid improvement in mean hemoglobin (≈1 g/dL), and maintenance in mean spleen volume (≈1700 cm^3) in both groups. Of 92 RUX patients who were not transfusion independent (TI) at W24, 46% became TI responders 12 weeks after MMB crossover. Post RUX→MMB, 23% achieved/maintained spleen response at W48. During the first two weeks post-RUX→MMB, 3% and 2% of patients reported new onset Grade 3/4 anemia and thrombocytopenia, respectively; no RUX discontinuation syndrome occurred; the risk of RUX-related weight gain did not increase after crossover.

In SIMPLIFY-2, W24 BAT→MMB was associated with a rapid improvement in mean hemoglobin level and stable/improved spleen volume. Total symptom score was improved by ≥50% at W24 in 22% of those who were on RUX before starting MMB in the study without washout.

Conclusion: Immediate transition from RUX to MMB in MF patients showed rapid anemia benefit without compromising safety, symptoms and splenic responses.

Encore abstract: Mesa R, *et al*. *Blood*. 2022;140 (suppl 1):3983-3985 (https://doi.org/10.1182/blood-2022-159636).

Updated results from the MOMENTUM phase 3 study of momelotinib versus danazol in symptomatic and anemic myelofibrosis patients previously treated with a JAK inhibitor

Perkins A[1], Gerds A[2], Mesa R[3], Vannucchi A[4], Al-Ali H[5], Lavie D[6], Kuykendall A[7], Grosicki S[8], Iurlo A[9], Goh Y[10], Lazaroiu M[11], Egyed M[12], Fox M[13], McLornan D[14], Yoon S[15], Gupta V[16], Kiladjian J[17], Donahue R[18], Kawashima J[18], Verstovsek S[19]

[1]Alfred Health,The Australian Centre For Blood Diseases,Monash University, Melbourne, Australia, [2]Cleveland Clinic Taussig Cancer Center, Cleveland, USA, [3]Wake Forest University School of Medicine, Winston Salem, USA, [4]University of Florence, Firenze, Italy, [5]University Hospital of Halle (Saale), Halle, Germany, [6]Hadassah University Medical Center, Jerusalem, Israel, [7]Moffitt Cancer Center, Tampa, USA, [8]Medical University of Silesia, Katowice, Poland, [9]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan , Italy, [10]Singapore General Hospital, Singapore, Singapore, [11]Policlinica de Diagnostic Rapid, Brasov, Romania, [12]Somogy County Kaposi Mór General Hospital, Kaposvár, Hungary, [13]Vall d'Hebron University Hospital, Barcelona, Spain, [14]Guy's and St Thomas' NHS Foundation Trust, London, UK, [15]Seoul St Mary's Hospital,College of Medicine,The Catholic University of Korea, Seoul, South Korea, [16]Princess Margaret Cancer Centre,University of Toronto, Toronto, Canada, [17]Université de Paris, AP-HP, Hôpital Saint-Louis,Centre d'Investigations Cliniques, Paris , France, [18]Sierra Oncology, Inc., San Mateo, USA, [19]The University of Texas MD Anderson Cancer Center, Houston, USA

Aim: To investigate the efficacy and safety of momelotinib (MMB) versus danazol (DAN) at Week 48 (W48) in JAK inhibitor (JAKi)-experienced patients with myelofibrosis (MF) from the international, phase 3 MOMENTUM study (Funding: Sierra Oncology, Inc., a GSK company; NCT04173494).

Method: Eligibility: Primary or post-ET/PV MF; DIPSS high risk, Int-2, or Int-1; Total Symptom Score (TSS) ≥10; hemoglobin <10 g/dL; platelets ≥25 x 10^9/L; prior JAKi for ≥90 days, or ≥28 days if red blood cell (RBC) transfusions ≥4 units in 8 weeks or Grade 3/4 thrombocytopenia/anemia/hematoma; palpable spleen ≥5 cm. Randomization: 2:1 to MMB 200 mg/day or DAN 600 mg/day for 24 weeks, followed by open-label (OL) MMB 200 mg/day. W48 endpoints: duration of responses (TSS, transfusion independence [TI], splenic), overall and leukemia-free survival (OS, LFS).

Results: At W48, 93/130 MMBàMMB and 41/65 DANàMMB patients received OL MMB. Of TSS responders, 1/32 MMBàMMB and 0/6 DANàMMB had TSS return ≥baseline; of TI responders, 4/40 and 3/13 required a RBC transfusion; of spleen responders, no patients had splenic volume ≥baseline. OS and LFS curves for both arms converged post-crossover to OL MMB (HR=0.945, 95% CI=0.528–1.693; HR=0.830, 95% CI=0.473–1.4555). The most common Grade ≥3 TEAEs in the OL phase were thrombocytopenia (MMBàMMB, 9%; DANàMMB, 15%) and anemia (MMBàMMB, 9%; DANàMMB, 2%); discontinuations occurred in 18% of MMBàMMB and 10% of DANàMMB. MMBàMMB patients with thrombocytopenia showed consistent efficacy and safety with the ITT set; patients with baseline platelets <50 x 10^9/L showed improved OS and LFS (HR=0.123; 95% CI=0.014–1.082 for both OS/LFS).

Conclusion: OL MMB maintained symptom, TI, and splenic responses with good safety in JAKi-experienced patients with MF, including anemic and thrombocytopenic populations.

Encore abstract: Gerds AT, *et al*. *Blood*. 2022;140 (suppl 1): 1514-1517 (https://doi.org/10.1182/blood-2022-162783).

Synergy between interferon and arsenic trioxide improved cytotoxic effect in JAK2-mutant MPN

Tursky M[1,2], Omari S[1,2], Wang K[1], Molloy T[1,2], Ma D[1,2]
[1]St Vincent's Hospital Sydney, Darlinghurst, Australia, [2]School of Clinical Medicine, St Vincent's Healthcare Clinical Campus, Faculty of Medicine and Health, UNSW Sydney, Sydney, Australia

Aim: JAK2-V617F is a frequent driver mutation in myeloproliferative neoplasms (MPN), and a subset of patients transform to secondary acute myeloid leukaemia (AML). Pegasys (pegylated interferon alpha-2a) is the preferred MPN treatment, but has low molecular response and ~20% discontinuation due to intolerance. Phenasen (arsenic trioxide) is a curative targeted therapy in acute promyelocytic leukaemia. Recent reports show arsenic trioxide potentiates interferon in MPN patient derived and murine progenitor cells *in vitro*, with >50% remission in the murine model (1). We hypothesised the combination treatment synergises in JAK2-V617F MPN cells, and aimed to assess Phenasen, Pegasys, and combination treatment on JAK2-V617F transformed MPN and control AML cell lines

Method: *In vitro* assessment of Phenasen, Pegasys and combination treatment with two JAK2-V617F MPN transformed and two JAK2-wildtype AML cell lines utilising proliferative (MTS) and leukaemic clonogenic cell (CFU) assays. An average of 11 independent replicates with ≥4-point dose curves were analysed with CompuSyn, a well-validated clinically-utilised mathematical determination of drug combination index and drug reduction potential.

Results: Synergy between Pegasys and Phenasen was observed in both JAK2-V617F transformed MPN cell lines and moderate synergy occurred in one control line. Combination treatment enabled a greater than 3 fold reduction in Pegasys dose and or Phenasen dose in JAK2-V617F transformed MPN to achieve the same proportion of aberrant cell death. This supports the hypothesis that combined treatment synergy improved cytotoxicity in JAK2-V617F MPN cells. Leukaemic clonogenic assays supported MTS data, showing Phenasen and Pegasys combination reduced aberrant cell proliferation and induced differentiation.

Conclusion: Combination Pegasys and Phenasen treatment showed greatest synergy in JAK2-V617F transformed MPN cell lines. The combination enabled a reduction in Pegasys and or Phenasen dose, which suggests therapeutic potential to achieve high clearance of aberrant cells with reduced side effects of individual compounds.

Reference: (1) Dagher T *et al.*, The Journal of Experimental Medicine, 2021.

Daratumumab addition for newly diagnosed AL amyloidosis results in rapid haematological response: outcome outside of clinical trials

Abro E[1], Anderson N[1], Tobin J[1], Mollee P[1]
[1]*Princes Alexandra Hospital, Brisbane, Australia*

Aim: The Andromeda study demonstrated benefit of upfront Daratumumab added to standard of care bortezomib, cyclophosphamide, dexamethasone (VCD) for newly diagnosed AL amyloidosis patients. We aim to evaluate and report the outcomes of the addition of Daratumumab to VCD (Dara-VCD) outside of clinical trials.

Method: Patient database between 2019 and 2023 with a confirmed new diagnosis of systemic AL amyloidosis and treated with Dara-VCD were compared to previously diagnosed and treated cohort. Demographic data, treatment received, disease characteristics, treatment response, and mortality were assessed.

Results: 126 patients consecutively treated were identified. 104 patients received VCD and 18 received Dara-VCD, with 67% males and 33% females. The involved light was lambda in 74% and kappa in 26%. Majority of patients had concurrent smouldering myeloma (69%) or monoclonal gammopathy (20%), with a minority diagnosed with symptomatic myeloma (11%).

At diagnosis both groups were equally matched for median age (65 vs 67 years), organ involvement cardiac (81% vs 78%), renal (73% vs 61%), number of organs involved (mean 2.4 vs 2.2), advanced Mayo 2004 cardiac stage (IIIa 18% vs 11%; IIIb 12% vs 17%). After 2 cycles of treatment response ≥ VGPR was 54% with VCD vs 72% with Dara-VCD (p=0.039). After 6 cycles of treatment response ≥ VGPR was 53% with VCD vs 64% with Dara-VCD (p=0.178). Prior to completing 2 cycles, 8 died in the VCD group and 3 died in the Dara-VCD group, with no further mortality in either group up to the completion of 6 cycles.

Conclusion: The addition of daratumumab to VCD in newly diagnosed AL Amyloidosis results in a significantly more rapid and deeper haematological response. Mortality is noted to occur early in the treatment course, and a rapid reduction in the pathogenic light chain is a priority to preserve organ function and minimise disease related mortality. Our data support the incorporation daratumumab to upfront AL amyloidosis treatment.

DVD treatment for myeloma – a regional centre's perspective

An T[1], Wong P[1]
[1]*Toowoomba Base Hospital, Toowoomba , Australia*

Aim: Multiple myeloma is a malignancy of plasma cells. Whilst it remains incurable, outcomes have significantly improved with newer combination therapies. Daratumumab, bortezomib and dexamethasone (DVD) was approved on the Pharmaceutical Benefits Scheme in Australia in January 2021 for treatment of myeloma at first relapse. Toowoomba is a major regional hospital in Queensland, Australia. Due to the tyranny of distance, this impacts on access to treatment, potentially affecting outcomes. We performed a retrospective study looking at patient outcomes treated with DVD at our institution.

Method: Patients with myeloma treated with DVD at first relapse were assessed. Baseline patient characteristics including demographics, time to first relapse, cytogenetics, best response and time to next relapse were collected. All patients received weekly bortezomib and subcutaneous daratumumab.

Results: Ten patients received DVD since 2021. Overall response rate was 70%, with 30% complete response, 10% very good partial response and 30% partial response. Two patients required dose reduction of bortezomib due to peripheral neuropathy with one patient requiring drug cessation. Median progression-free survival was 12 months. Four patients have experienced disease progression and have changed to subsequent lines of therapy. Two patients died due to non-myeloma related causes.

Conclusion: Compared to the 27 months progression free survival stated in the CASTOR study, our patients had a poorer response to DVD used at first relapse in terms of overall response and progression-free survival. High risk myeloma, cessation of bortezomib and early relapse following first line therapy had a negative impact on treatment response. Larger datasets are required to assess whether weekly rather than twice weekly bortezomib significantly impacts overall response and long term outcomes. Whilst DVD is a feasible treatment option, better triplet therapy combinations should be considered at first relapse.

AL Amyloidosis - MM24

Attreya A[1], Gibbs S[1], Jaccard A[1], Ting S[1], Motorna O[1], Horvath N[1], Mollee P[1], Sidiqui H[1], Roussel M[1]
[1]*Eastern Health, Briar Hill , Australia*

Background: Systemic AL amyloidosis is caused by the deposition of misfolded monoclonal immunoglobulin free light chains (sFLC) in various organs. Treatment of AL amyloidosis relies mainly on chemotherapy aimed at suppressing the underlying secreting plasma cell clone. Organ responses and survival are greatly influenced by the degree of hematological response evaluated by the decrease in sFLC. Over the last 5 years, antiCD38 monoclonal antibodies (mAb), such as daratumumab (DARA), have emerged as breakthrough targeted therapies for pts with multiple myeloma (MM). CD38, is a transmembrane glycoprotein that functions both as a signal-transducing receptor and a multifunctional ectoenzyme. Its expression is increased in MM and AL amyloidosis plasma cells. DARA received approval in combination with CyBorD in frontline AL amyloidosis (1). In the relapse setting, DARA demonstrated a good efficacy and safety profile (2,3) but its activity could be enhanced with IMiD®, since it could increase the expression of CD38 levels on plasma cells. In AL amyloidosis, various groups demonstrated that pomalidomide (POM) is very effective and better tolerated than lenalidomide, especially in pts with renal insufficiency (4,5) with no dose modification (4 mg). Combining an antiCD38 mAb to POM could therefore be an attractive regimen for relapsed pts. Isatuximab (ISA) is another antiCD38 mAb that binds selectively to a unique epitope on the CD38 receptor and has been approved in RRMM in combination with POM or carfilzomib plus low dose dexamethasone (DEX) (6,7) with a good safety profile.

Objective: The aim is to evaluate the efficacy and safety of the combination of ISA, POM, and DEX in pts with AL amyloidosis who did not reach at least very good partial response (VGPR) and/or who have relapsed.

Material & Methods: In this multicentre Australian and French, single-arm, phase 2 study, we planned to include 46 previously treated pts. Main inclusion criteria comprise: ≥1 previous line of therapy without VGPR/CR at time of inclusion; measurable haematological disease: dFLC > 50 mg/L; symptomatic organ involvement; adequate bone marrow and organ functions; no dialysis; no overt MM, no cardiac stage IIIb pts; ECOG <2; no previous antiCD38 or POM therapy (if refractory to POM). Pts eligible to enter the study will receive 28-days cycles of ISA (10 mg/kg, IV, weekly for 1st cycle then every-other-week), POM 4 mg (days 1-21) and DEX (10-20 mg, weekly). The treatment period will be 12 months, unless CR at the completion of 9 cycles, disease progression or unacceptable toxicity occurs. The primary endpoint will be ≥VGPR at the completion of 6 cycles. Secondary endpoints will be: overall haem response rates at various time points; progression-free survival; organ response rates at 1 year; overall survival; time to haem and organ responses; safety and tolerability; and quality of life (EQ-5D-3L). Exploratory endpoints will comprise: impact of t(11.14) on responses; minimal residual disease by NGS and by mass spectrometry.

Results: As of April 2023, 33 patients were screened: 26 are receiving therapy, 1 is in screening and 6 is screen fail. To date, no unexpected toxicities were reported. The data safety and monitoring board meetings will be called every 6 months or whenever indicated.

Conclusion: This is the first prospective study of ISA in combination with POM and DEX in AL amyloidosis. Patients characteristics will be updated and preliminary results and safety data will be reported during the Blood Congress.

References

1. Kastritis E et al, NEJM
2. Roussel M et al, Blood. 2020 Apr 30;135(18):1531-1540
3. Sanchorawala V et al, Blood. 2020 April 30, 135 (18): 1541–1547.
4. Palladini G et al, Blood. 2017 Apr 13;129(15):2120-2123
5. Sharpley FA et al, Br J Haematol. 2018 Nov;183(4):557-563
6. Attal M et al; ICARIA-MM study group, Lancet. 2019 Dec 7;394(10214):2096-2107
7. Moreau P et al, Lancet. 2021 Jun 19;397(10292):2361-2371

An uncommon case of Crystal Laden Histiocytosis associated with a Plasma Cell Dyscrasia

Baskaran A[1], Aung W[1], Tong M[1], Motum P[1], Hua M[1]
[1]*Liverpool Hospital , Sydney , Australia*

Crystal-storing histiocytosis (CSH) is a rare entity associated with monoclonal gammopathies which may precede or occur in conjunction with plasma cell dyscrasias, lymphoproliferative disorders (LPD), and rare inflammatory conditions. It is characterized by intra-lysosomal accumulation of immunoglobulins as crystals, and predominantly associated with kappa light chain restriction[1].

We present a rare case of an 81-year-old female with bone marrow evidence of prominent crystal-storing histiocytes associated with longstanding MGUS with a small IgG kappa paraprotein and notable kappa light chain burden without myeloma defining events or evidence of a lymphoproliferative disorder. She was diagnosed with MGUS ten years ago with an elevated IgG Kappa paraprotein of 9.9g/L and notable kappa light chain burden ranging fluctuating between 1200 to 3000mg/L. Screening investigations did not identify myeloma-defining events including preserved haematological and biochemical parameters and no lytic lesions on the CT skeletal survey and no evidence of mass lesions or adenopathy on imaging. Her BMAT showed a prominent population of crystal-storing histiocytes with 8% plasma cell population. Cytogenetic analysis demonstrated hyperdiploid with segmental copy number abnormalities (loss of 1p, 13, and 20q) which are common recurrent cytogenetic abnormalities seen in multiple myeloma.

This case describes the rare entity of crystal-storing histiocytosis associated with MGUS which has run an indolent course of 10 years to date. CSH can be associated with a variety of conditions including LPD, MM, and rare inflammatory conditions, with variable clinical course and prognosis that can in some cases be associated with a more aggressive biology and course.

Reference:

1. Mobarki, M., et al., *Crystal-Storing Histiocytosis: The Iceberg of More Serious Conditions.* Diagnostics, 2023. **13**(2): p. 271.

Small nucleotide, copy number and structural variants cooperate to hijack driver genes in extramedullary progression of myeloma

Bingham N[1], Shah J[2], Wong D[1,2], Reale A[1,2], Khong T[1,2], Mithraprabhu S[1,2], Spencer A[1,2]
[1]Alfred Health, Melbourne, Australia, [2]Monash University, Melbourne, Australia

Aim: Extramedullary disease (EMD) affects 30% of multiple myeloma (MM) patients, predicting poor survival due to aggressive kinetics and drug resistance. Understanding EMD genomics may inform targeted treatment approaches.

Method: EMD samples from n=15 obtained (n=8 fresh, n=7 formalin-fixed, paraffin embedded). Second biopsies taken in n=3. Buccal swabs excluded germline variants. DNA extraction: QIAGEN DNeasy kit. Whole genome sequencing performed (30x; xGen PRISM library preparation; Illumina Novaseq).

Bioinformatics: short nucleotide variants (SNVs), copy number variations (CNVs) and structural variants (SV) identified using Broad Institute GATK, CNVpytor and Manta, respectively. Droplet digital PCR for validation.

Results: Median age at MM diagnosis: 52 years (n=4 primary, n=11 secondary EMD). 46% hyperdiploid.

Driver mutations (DM) in MAPK pathway identified in 80%, primarily codon 61 of *NRAS* and *KRAS* (n=5, n=3); 2 non-p.Q61 mutations seen. n=3 activating *BRAF* mutations. 26.6% had loss of function *TP53* mutations.
Those with no MAPK DM had median total SNV of 77,142, with a tumour mutational burden (TMB) of 15 mutations/Mb, compared to 20,543 and 3.2 in DM patients.
CNV/SV analysis identified gain(3q), gain(1q), del(1p) and del(13q) in 93%, 86%, 46% and 73% respectively. Gains of *BRAF* (66%) and *MYC* (53%) and loss of *TP53* (40%) were frequent; with SNV, 20% had biallelic loss. Secondary translocations seen in 40% of patients, involving *MYC*, *FGFR3*, *CCND2* and *CCND3* in 20%, 13%, 6% and 6%, respectively. Partner genes *IGH*, *IGL* and *TXNDC5*. Median of 44 SV/sample. Most SV were not identified earlier in disease, frequently involved MM driver genes/super-enhancers.
Sequential biopsies demonstrated temporospatial persistence of DM: same DM detected at different anatomical sites. CNV/SV increased with relapse, consistent with role in disease progression and drug resistance.

Conclusion: MAPK DM, high TMB and genomic instability suggest roles for MAPK-targeted therapies, immunotherapies and DNA damage repair inhibitors, respectively. Recurrent codon 61 mutations in RAS suggest a specific role in EMD progression.

Functional screening identifies non-muscle myosin heavy chain IIA (MYH9) as novel cereblon (CRBN) interactor via BioID2-dependent proximity labelling

Costacurta M[1,2], Sandow J[3,4], Maher B[1,2], Susanto O[1,2], Vervoort S[3], Devlin J[5,6], Garama D[7,8], Condina M[9,10], Steele J[11,12], Kahrood H[11,12], Gough D[7,8], Johnstone R[5,6], Shortt J[1,2,5,6]
[1]Monash Haematology, Monash Health Clayton, Clayton, Australia, [2]Blood Cancer Therapeutics Laboratory, School of Clinical Sciences at Monash Health, Monash University, Clayton, Australia, [3]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [4]Department of Medical Biology, The University of Melbourne, Parkville, Australia, [5]Translational Haematology Program, Peter MacCallum Cancer Centre, Melbourne, Australia, [6]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Australia, [7]Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, Australia, [8]Department of Molecular and Translational Science, Monash University, Clayton, Australia, [9]Mass Dynamics, Melbourne, Australia, [10]Clinical & Health Sciences, University of South Australia, Adelaide, Australia, [11]Monash Proteomics and Metabolomics Platform, Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia, [12]Monash Bioinformatics Platform, Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia

Aim: Immunomodulatory imide drugs (IMiDs) are a key therapy for multiple myeloma (MM). IMiDs bind cereblon (CRBN), the receptor for the Cullin Ring Ligand 4 ubiquitin-ligase complex, redirecting its substrate specificity towards neosubstrates Ikaros (IKZF1), Aiolos (IKZF3), leading to MM cell death. Despite the availability of new generation IMiDs, most patients ultimately develop IMiD-resistant disease. Moreover, mechanisms of distinct and overlapping IMiD toxicities remain poorly defined. We aimed to define the IMiD-CRBN interactome using a novel proximity labelling approach to further inform mechanisms of IMiD activity and toxicity.

Methods: We generated a chimeric protein fusing CRBN with BioID2 (BioID2-CRBN), a bacterial biotin ligase that biotinylates proximal lysine residues. Cells expressing BioID2-CRBN were treated with IMiDs (lenalidomide, pomalidomide and iberdomide) ± bortezomib, followed by liquid chromatography mass spectrometry (LC-MS) analysis of the biotinylated proteome. Western blotting was employed for low-throughput validation of 'hits' from the BioID2 screen. MaxQuant (v1.6.17) and RStudio 4.2 were adopted for data analysis.

Results: BioID2-CRBN induced biotinylation of known CRBN interactors and substrates (COP9 signalosome units, CUL4, HSP70), and of known CRBN-IMiDs neosubstrates (IKZF1/3 and CK1a). Additionally, we identified non-muscle myosin heavy chain IIA (MYH9) as a novel CRBN interactor and that IMiD treatment augments this interaction without subsequent MYH9 degradation. Analysis of publicly available ubiquitin-MS datasets also indicates that MYH9 is ubiquitinated at K821 following lenalidomide treatment.

Conclusion: MYH9 is a novel CRBN interactor that may be ubiquitinated in the presence of IMiDs. We posit that IMiD-augmented ubiquitination of MYH9 may modify protein function with potential mechanistic implications for thrombopoiesis, thrombosis and immune effector cell motility.

No conflict of interest to disclose.

Ectopic Amylase Production as a Marker of Disease Activity in a Patient with Oligosecretory Relapse of IgG-kappa Multiple Myeloma

Gardiner S[1], Quach H[1,2]
[1]St Vincent's Hospital Melbourne, Fitzroy, Australia, [2]Department of Medicine, University of Melbourne , Melbourne, Australia

This case report describes a 72-year-old South Asian man with heavily pre-treated R-ISS II IgG-kappa multiple myeloma identified to have an elevated serum amylase at the time of recent disease relapse. Extensive investigations did not identify a pancreatic cause for the hyperamylasaemia and with commencement of therapy both a reduction in serum paraprotein and serum amylase was observed. Ectopic production of serum amylase by multiple myeloma has been well documented and is associated with a high tumour burden, presence of extramedullary disease and reduced overall survival. As yet it has not been found to clearly correlate with a particular heavy or light chain isotype nor presence of high risk cytogenetic features. Given it's rarity it is unlikely to be used as a prognostic marker on a large scale however it remains an important prognostic marker in patient's whose myeloma demonstrates ectopic amylase production. In this case following an initial fall in serum paraprotein and amylase in response to treatment, the patient's amylase again began to rise. Oligosecretory relapse of disease was confirmed when a subsequent PET scan confirmed progressive disease despite the patient's serum paraprotein remaining stable. This is the first time ectopic amylase production has been described as a sensitive marker of disease activity in a patient with oligosecretory relapse. In addition to contributing to the prognostic evidence base regarding ectopic amylase production, it suggests that in cases where ectopic amylase production has been demonstrated it may be a more sensitive marker of disease progression than serum paraprotein.

Transient hyperphosphatasemia in an adult with myeloma: a case report

Goh K[1], Trambas C[2], Quach H[1,3]

[1]St Vincent's Hospital Melbourne, Fitzroy, Australia, [2]St Vincent's Pathology, Fitzroy, Australia, [3]University of Melbourne, Parkville, Australia

Abstract: Transient hyperphosphatasemia (TH) is characterised by isolated elevation of serum alkaline phosphatase (ALP), usually seen in infants or young children and rarely reported in adults. This benign phenomenon has no identifiable liver or bone disease, and ALP levels generally return to normal within a few months. We report a case of TH in a 74-year-old woman with multiple myeloma which was detected on routine blood tests. Her myeloma, currently in partial remission on Pomalidomide therapy, was diagnosed 3 years prior during work-up of locally advanced breast cancer (now in remission post-treatment). Laboratory analyses revealed a sudden dramatic rise in ALP level to 1214 U/L, a 10-fold increase relative to the upper limit of the normal reference interval. The remainder of the liver function panel was normal. The patient lacked relevant symptoms or clinical signs. This isolated ALP increase was initially concerning for a malignant bone process given her history of myeloma and breast cancer. However, CEA and CA-15-3 were negative, breast mammogram and ultrasound showed no recurrence of breast cancer, whole body imaging including PET scan showed no FDG-avid lesions, and paraprotein was stable. ALP isoenzyme electrophoresis results showed a pattern consistent with TH. The ALP levels spontaneously improved to her baseline level of 159 U/L after 1 month. TH in myeloma patients is a benign phenomenon, but it is important to differentiate from other potential causes of ALP elevation including bone and liver diseases. Interestingly, myeloma patients have been reported to have lower ALP levels compared to those with bone metastases due to solid tumours, and an ALP rise may even predict positive response to therapy. Although rare in adults, we recommend that clinicians are aware of the diagnosis of TH as this may save over-investigation. ALP isoenzyme electrophoresis is useful in such instances.

First Phase 3 results from CARTITUDE-4: Cilta-cel versus Standard of Care (PVd or DPd) in Lenalidomide-Refractory Multiple Myeloma

Dhakal B[1], **Harrison S[2]**, Yong K[3], Mateos M[4], Moreau P[5], van de Donk N[6], Sidana S[7], Popat R[8], Lendvai N[11], Lonardi C[9], Slaughter A[10], Schecter J[11], Li K[12], Zudaire E[12], Chen D[13], Gilbert J[14], Pacaud L[15], Patel N[15], San Miguel J[16], Einsele H[17]

[1]Medical College of Wisconsin, Milwaukee, USA, [2]Peter MacCallum Cancer Centre, Melbourne, Australia, [3]University College London Cancer Institute, London, UK, [4]University Hospital of Salamanca/IBSAL/CIC/CIBERONC, Salamanca, Spain, [5]Hematology Clinic, University Hospital Hotel-Dieu, Nantes, France, [6]Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [7]Stanford University School of Medicine, Stanford, USA, [8]University College London Hospitals, NHS Foundation Trust, London, UK, [9]Janssen, Buenos Aires, Argentina, [10]Cilag GmbH International, Zug, Switzerland, [11]Janssen Research & Development, Raritan, USA, [12]Janssen Research & Development, Spring House, USA, [13]Janssen Research & Development, Shanghai, China, [14]Janssen Research & Development, High Wycombe, UK, [15]Legend Biotech USA Inc., Somerset, USA, [16]University of Navarra, Pamplona, Spain, [17]Universitätsklinikum Würzburg Medizinische Klinik und Poliklinik II, Würzburg, Germany

Aim: CARTITUDE-4 is a phase 3 trial of ciltacabtagene autoleucel (cilta-cel), a dual-binding, BCMA-targeting CAR-T cell therapy, vs standard of care (SOC) in lenalidomide-refractory patients.

Methods: 419 patients with 1-3 prior lines of therapy (LOT), including a PI and IMiD, were randomised to apheresis then bridging therapy (pomalidomide, bortezomib, and dexamethasone [PVd] or daratumumab, pomalidomide, and dexamethasone [DPd]) following by 1 cilta-cel infusion or SOC where they received PVd or DPd until progression. The primary endpoint was progression-free survival (PFS).

Results: 176 patients received cilta-cel as study treatment, 20 received it after progressive disease (PD) on bridging therapy, and 208 received SOC (PVd, n=28; DPd, n=183). At Nov 1, 2022, data cut-off, median follow-up was 16 months (range, 0.1–27). Primary endpoint was met; cilta-cel reduced risk of progression/death by 74% (HR=0.26; P<0.0001). Cilta-cel significantly improved overall response rate (ORR), rate of ≥complete response (CR), and overall MRD-negativity rate vs SOC (Table 1), with a positive trend in overall survival (HR=0.78; 95% CI, 0.5-1.2). 97% and 94% of patients treated with cilta-cel or SOC had grade 3/4 AEs, respectively, including infections (27% vs 25%) and cytopenias (94% vs 86%). 39 and 46 patients died in the cilta-cel and SOC arms, respectively (14 and 30 due to PD). 76% of patients receiving cilta-cell as study treatment had cytokine release syndrome (1% grade 3; no grade 4/5) and 5% had immune effector cell associated-neurotoxicity syndrome (all grade 1/2). 1 patient had a grade 1 movement/neurocognitive treatment-emergent adverse event.

Conclusions: A single cilta-cel infusion significantly improved PFS vs SOC in lenalidomide-refractory patients with 1-3 prior LOT, with a favorable benefit/risk profile across patient populations. The 74% reduction in progression/death and high rates of CR and MRD-negativity highlight the potential for cilta-cel to become a key therapy for patients with MM after first relapse.

Table 1: Cilta-cel vs SOC outcomes (ITT)

	Cilta-cel (n=208)	SOC (n=211)	HR[a]	Odds ratio
Median PFS, months (95% CI)	NE (23–NE)	12 (10–14)	0.26 (0.18–0.38) (P<0.0001)	
12-month PFS, % (95% CI)	76 (69–81)	49 (42–55)		
ORR, n (%)[b]	176 (85)	142 (67)		3 (P<0.0001)
≥CR[b]	152 (73)	46 (22)		10 (P<0.0001)
10^{-5} MRD negative,[c] n (%)	126 (61)	33 (16)		9 (P<0.0001)

[a]Per computerised algorithm by constant piecewise weighted log-rank test.
[b]In 176 patients who received cilta-cel as study treatment: ORR, 175 (99%); ≥CR, 152 (86%).
[c]For MRD-evaluable patients: cilta-cel, 88% (126/144); SOC, 33% (33/101).

Novel CAR-T cell therapy targeting kappa myeloma antigen for the treatment of multiple myeloma

Oliaro J[2,3], Li J[2], Haynes N[2], Hilton H[4], Dunn R[4], **Harrison S[1,2,3]**
[1]Clinical Haematology, Peter Maccallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia, [2]Centre of Excellence in Cellular Immunotherapy, Peter MacCallum Cancer Centre, , , Melbourne, Australia, [3]Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia, [4]HaemaLogiX Ltd, Sydney, Australia

Introduction: Multiple myeloma (MM), the second most common blood cancer, is characterized by the accumulation of malignant plasma cells in the bone marrow. Chimeric Antigen Receptor (CAR)-T cell therapy has recently entered the standard of care for relapsed and refractory MM, following the recent FDA-approval of two CAR-T cell products, ide-cel® and cilta-cel®, which target the B cell maturation antigen (BCMA). However, despite impressive response rates, most patients relapse within 1-3 years, highlighting the need to develop novel CAR targets for this disease indication.

Methods: Kappa (κ) myeloma antigen (KMA) is a tumour specific membrane associated protein expressed on malignant plasma cells in patients with kappa light-chain restricted (κ-type) MM. KMA is absent on normal plasma cells and haematopoietic stem cells, making it an attractive and alternative target antigen for CAR-T cell therapy for MM. The monoclonal antibody, KappaMab (MDX-1097), binds to a conformational epitope on KMA, and has been assessed in phase I, IIa and IIb clinical trials in relapse refractory myeloma patients. Here, we have engineered a lentiviral vector encoding a second-generation CAR expressing a scFv from MDX-1097, fused to a 4-1BB co-stimulatory domain and CD3 zeta chain, to test in preclinical models of MM.

Results: We successfully generated human anti-KMA CAR-T cells with high and stable CAR expression and a predominately memory T cell phenotype. The CAR-T cells selectively killed KMA-expressing tumour lines and secreted interferon-gamma upon target recognition. Futhermore, a single dose of anti-KMA CAR-T cells demonstrated potent anti-tumour activity in a xenograft model. All mice treated with a 5e6 CAR-T cell dose were alive at 100 days post-treatment, had persisting circulating CAR-T cells and no evidence of disease.

Conclusion: Our data demonstrates that anti-KMA CAR-T cell therapy is a novel and potent treatment ready to enter a phase I clinical trial for patients with multiple myeloma.

Using digital health to empower Multiple Myeloma patients in shared decision-making: Findings from the ChoiceApp® pilot study

Fifer S[1], Beer H[2], Combe N[2], Axford L[1], Godsell J[1], **Joshi M[1]**, Monro D[1], Toh L[1]
[1]Community and Patient Preference Research Pty Ltd, Sydney, Australia, [2]Myeloma Foundation of Australia Inc, Burnely, Australia

Aim: Australia is embracing a patient-centred approach to healthcare, which includes the implementation of shared decision-making (SDM)[1]. SDM involves considering patient values, preferences, and circumstances throughout the treatment journey[2]. Understanding an individual's preferences is important given there is significant heterogeneity when it comes to what treatment attributes people with MM value[3]. The ChoiceApp® pilot study was conducted to investigate the effectiveness of a digital tool in empowering patients to identify and communicate their personal treatment priorities to their HCP, thus promoting SDM.

Method: The pilot involved 5 haematologists and 14 MM patients in Australia. Using ChoiceApp®, patients completed two 5-10minute surveys: 1) a best worst scaling (BWS) exercise on treatment preferences; 2) an adapted version of the MYPOS QoL questionnaire. Upon completion, they received a real-time personalised report summarising their results and were asked to share this report with their HCP in their next consultation. Patients and HCPs shared their experiences with the research team during individual in-depth interviews.

Results: Qualitative data suggests ChoiceApp® can enhance SDM by providing confirmation about what matters most to patients and building their confidence in discussing these matters with their HCPs. The tool appeared particularly useful for patients who had less involvement in decision-making, or understanding of their disease (e.g., newly diagnosed, lower confidence in clinical environment) or those undergoing a change in treatment. Feedback from pilot participants was used to refine ChoiceApp® and enhance user experience. An updated version of ChoiceApp® has been launched to the broader MM community and early data from this next phase will be presented in the oral/poster presentation.

Conclusion: Digital tools like ChoiceApp® can be effective in assisting MM patients and their HCPs to discuss individual treatment preferences and is particularly valuable at specific points in the treatment pathway. Longitudinal follow-up is required to determine extent of impact on SDM outcomes.

References:

- Australian Commission on Safety and Quality in Health Care. National Safety and Quality Health Service Standards. 2nd ed. Updated May 2021. Sydney: ACSQHC; 2021. Available from: https://www.safetyandquality.gov.au/sites/default/files/2021-05/national_safety_and_quality_health_service_nsqhs_standards_second_edition_-_updated_may_2021.pdf

1. Hoffman T, Legare F, Simmons M, McNamara K, McCaffery K, Trevena L, et al. Shared decision making: what do clinicians need to know and why should they bother? Med J Aust 2014;201(1):35–9.
2. Fifer SJ, Ho KA, Lybrand S, Axford LJ, Roach S. Alignment of preferences in the treatment of multiple myeloma – a discrete choice experiment of patient, carer, physician, and nurse preferences. *BMC Cancer*. 2020;20(1):546. doi:10.1186/s12885-020-07018-6

Inferior outcomes in t(11;14) multiple myeloma: a report from the Australian Lymphoma Leukaemia Group (ALLG) and the Australian Myeloma and Related Diseases Registry (MRDR)

Lim K[1], Wellard C[2], Talaulikar D[3], Tan J[4], Loh J[5], Murphy M[6], Kuzich J[7,8], Puvanakumar P[8], Ho M[3], Zeglinas N[1], Low M[5], Routledge D[8], Lim A[7], Gibbs S[6], Quach H[1], Morgan S[4], Moore E[2], Ninkovic S[1]
[1]St Vincent's Hospital Melbourne, , Australia, [2]School of Public Health and Preventive Medicine, Monash University, , Australia, [3]The Canberra Hospital, , Australia, [4]The Alfred Hospital, , Australia, [5]Monash Health, , Australia, [6]Eastern Health, , Australia, [7]Austin Health, , Australia, [8]Peter MacCallum Cancer Centre and Royal Melbourne Hospital, , Australia

Aim: The presence of t(11;14) in Multiple Myeloma (MM) has emerged as predictive for response to BCL2 inhibitors with ~20% of patients having t(11;14) as a primary cytogenetic event at first diagnosis. We aimed to explore the prognostic impact of t(11;14) in MM patients on treatment patterns and outcomes in Australia.

Method: The Australian Lymphoma Leukaemia Group (ALLG) embarked on a retrospective, observational study using real-world data in 74 MM patients (3/74 with coexisting hyperdiploidy) treated across 7 centres with t(11;14) [t(11;14)-MM] diagnosed between January 2009 and December 2019. This was compared to 159 patients with high-risk IgH translocations (IgH HR-MM) and 111 patients with hyperdiploidy but no IgH translocation (Hyperdiploid-MM) from the Australian Myeloma and Related Diseases Registry (MRDR).

Results: Amongst the groups, no appreciable differences in age, gender, ISS, LDH levels and treatment patterns (1st, 2nd and 3rd line) were observed. No significant differences in co-existing 1q21 gain/amp or del(17p) prevalence were detected [1q21 gain/amp: 14%, 14% and 20% (p=0.36) and del(17p) 12%, 9% and 7% (p=0.54)]. Median PFS-1 was not significantly different between groups. However, t(11;14)-MM and IgH HR-MM had an inferior PFS-2 vs hyperdiploid-MM (median PFS-2 8.2, 10.0 and 19.8 months respectively; p=0.002) . The 3-year OS were 69%, 71% and 82% respectively (p=0.026). In the t(11;14)-MM group, gain or amplification of 1q21 at diagnosis (n=9) predicted for poorer OS (HR 3.46, 95% CI 0.93-12.02; p=0.002). Eleven patients had received Venetoclax (median 3 prior lines) with 45% achieving a very good partial response or better.

Conclusion: These results provide insight on the prognostic impact of the primary genetic events in MM and suggest survival outcomes of t(11;14) MM to be inferior to MM with hyperdiploidy but comparable to that of the high risk IgH mutations, particularly in the relapsed refractory setting. As such, Bcl2 inhibtors such as Venetoclax which are highly effective in patients harbouring t(11;14) should be explored earlier in the treatment algorithm.

Daratumumab, Bortezomib and Dexamethasone for first relapse of multiple myeloma in the real world- a retrospective analysis from a single Australian institution

Mckeague S[1], Weber N[1]
[1]*Royal Brisbane & Women's Hospital, Brisbane, Australia*

Aim: Daratumumab, bortezomib and dexamethasone (DVD) has become a commonly used second-line therapy for myeloma in Australia, based on subgroup analysis from the CASTOR registration trial that showed a progression free survival (PFS) of 27 months in this group(1). This study aimed to examine local use of DVD and to determine the efficacy and toxicity in a "real-world" setting.

Method: We retrospectively reviewed patients treated with DVD at first relapse from a single, tertiary Australian haematology centre between 1/1/21 and 1/3/23. The primary outcomes were overall response rate (ORR) and PFS. Secondary outcomes were overall survival (OS), PFS for different subgroups, time to next treatment (TTNT), mode of delivery and adverse events.

Results: Thirty-nine (n= 39) patients were included for analysis. Most patients were treated with weekly bortezomib (89%) and subcutaneous daratumumab (59%). When compared with CASTOR, the real-world population was older (median 71 vs. 63) with a higher prevalence of high-risk cytogenetics (33% vs. 23%), bortezomib exposure (79% vs. 52%), bortezomib refractoriness (12% vs.0%), lenalidomide exposure (58% vs. 12%) and lenalidomide refractoriness (51% vs. 5%)(2). The ORR was 48.7% and the median PFS was 8.33 months, which compares unfavourably with CASTOR (92% and 27mo, respectively)(1). OS was not met at a median follow up of 12.73 months. Within our cohort, median PFS was significantly shorter in those with high-risk cytogenetics (4.67 vs. 18.7 months, p = 0.009).

Conclusion: The impressive PFS seen with DVD in first relapse in the CASTOR registration trial is not replicated in the real world. "Real-life" myeloma patients are older, more high-risk, have more drug exposure and more bortezomib/lenalidomide resistance. The modest efficacy of DVD is of particular relevance to Australian practice, where local restrictions on drug access can result in this regimen being used indiscriminately in subgroups who may benefit from alternate therapy.

References:

1. Mateos MV, Sonneveld P, Hungria V, Nooka AK, Estell JA, Barreto W, et al. Daratumumab, Bortezomib, and Dexamethasone Versus Bortezomib and Dexamethasone in Patients With Previously Treated Multiple Myeloma: Three-year Follow-up of CASTOR. Clin Lymphoma Myeloma Leuk. 2020 Aug;20(8):509–18.

2. Palumbo A, Chanan-Khan A, Weisel K, Nooka AK, Masszi T, Beksac M, et al. Daratumumab, Bortezomib, and Dexamethasone for Multiple Myeloma [Internet]. https://doi.org/10.1056/NEJMoa1606038. 2016 [cited 2019 Nov 17]. Available from: https://www.nejm.org/doi/10.1056/NEJMoa1606038?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dwww.ncbi.nlm.nih.gov

Identifying early suboptimal haematological response in patients with AL amyloidosis treated with bortezomib-based chemotherapy

Gibbons A[1], Ranjit Anderson N[1,2], Sidiqi H[3,4], Gibbs S[5,6,7], Korczyk D[1,2], Mollee P[1,2]
[1]Queensland Amyloidosis Centre, Princess Alexandra Hospital, Woolloongabba, Australia, [2]School of Medicine, University of Queensland, Brisbane, Australia, [3]Fiona Stanley Hospital, Perth, Australia, [4]Curtin Medical School, Curtin University , Perth, Australia, [5]Eastern Health Monash University Clinical School, Melbourne, Australia, [6]Epworth Freemasons, Melbourne, Australia, [7]Alfred Health, Melbourne, Australia

Aim: We aimed to determine in impact of haematological response after 2 months of bortezomib-based therapy on subsequent best haematological and organ responses and survival.

Method: Subjects had a histological diagnosis of AL amyloidosis and initial treatment with a bortezomib-based regimen. The impact of haematological response after 2 months of treatment on subsequent best haematological and renal response was assessed using Fisher's exact test and on survival was assessed by landmark analysis using log-rank or Cox regression analysis.

Results: 150 patients with AL amyloidosis were identified: Median age was 66 yrs and 35% were male. 75% of cases were lambda restricted and the median dFLC was 160mg/L. Cardiac stage was 1(13%), 2(55%), 3A(17%) and 3B(15%) and 71% had renal involvement. Median OS was 6.2 years.

Haematological response after 2 months of bortezomib-based induction was: CR (17%), VGPR (40%), PR (17%) and less than PR (16%). 10% of patients died prior to 2 months. Failure to achieve PR by 2 months was associated with only 5% going on to achieve VGPR or better ($p<0.001$). Similarly, failure to achieve PR by 2 months predicted a low likelihood of improving organ function with less chance of cardiac (6% vs 47%, $p=0.001$) and renal (20% vs 41%) responses. In a landmark analysis, failure to achieve PR after 2 months predicted worse OS (median 48 vs 93 months, $p=0.041$), a finding confirmed in multivariate analysis. This effect was particularly evident for patients with cardiac stage 3A and 3B disease where failure to achieve early PR was associated with a median OS of 7 vs 70.

Conclusion: Failure to achieve haematological PR after 2 months of bortezomib-based therapy is associated low likelihood of subsequently achieving deep haematological response, organ responses and predicts poor survival. Such patients should be switched to alternate salvage therapy.

More efficient delivery of high-cost standard-of-care therapies in relapsed multiple myeloma using real-time feedback of patient-reported outcome measures: the MY-PROMPT-2 trial

Moore E[1], Harrison S[2], Ho P[3,4], Irving A[1], King T[3,6], McCaughan G[7], McQuilten Z[1], Mollee P[9,10], Park S[11], Petrie D[8], Reynolds J[5,6], Rutherford C[12], van Tonder T[1], Wellard C[1], Wood E[1], Spencer A[5,6]

[1]School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia, [2]Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia, [3]Royal Prince Alfred Hospital, Sydney, Australia, [4]University of Sydney , Sydney, Australia, [5]Central Clinical School, Monash University, Melbourne, Australia, [6]The Alfred Hospital, Melbourne, Australia, [7]St Vincent's Hospital , Sydney, Australia, [8]Centre for Health Economics, Monash University, Melbourne, Australia, [9]Princess Alexandra Hospital , Brisbane, Australia, [10]University of Queensland, Brisbane, Australia, [11]School of Medical Sciences, University of Sydney, Sydney, Australia, [12]Cancer Care Research Unit, Faculty Medicine and Health, University of Sydney, Sydney, Australia

Many patients on the ANZ Myeloma and Related Diseases Registry stop standard-of-care (SoC) therapy prematurely. This impaired duration on therapy (DoT) reduces the potential survival benefit. We hypothesise that if clinicians were made aware of emerging symptoms, DoT could be optimised, which would enhance treatment effectiveness. MY-PROMPT-2 builds on our pilot MY-PROMPT RCT that confirmed feasibility and acceptability of real-time patient reported outcome measure (PROM) feedback to clinicians.

In patients with relapsed MM (RMM), receiving SoC lenalidomide, carfilzomib or daratumumab-based therapies, we aim to determine whether routine real-time PROMs feedback to clinicians at patient visits improves event-free survival (EFS: time from randomisation to an event [permanent discontinuation of treatment, progression or death]) compared to patients on SoC alone.

Methods: This parallel, non-blinded, multicentre Bayesian RCT, uses 1:1 allocation, stratified by the 3 SoC regimens and age, with provision to recruit 200 adults.
Intervention: PROM results summary fed back to clinicians at monthly visits for 12 months. PROMs:

- MyPOS: MM-specific, 30 items - symptoms/ mood/ healthcare support
- further SOC regimen-specific questions (≤5) for common side effects

ePROM system: REDCap-based for easy implementation. PROMs are emailed to intervention patients 1 week before visits. A summary is emailed to clinician, patient, and site staff.
PROMs comparing health-related quality of life (EORTC QLQ-C30) and treatment satisfaction (TSQM-9) are collected 3-monthly in both arms for 12 months.
Novel statistical trial design: Once ≥60 events have been observed between the 2 arms, EFS monitoring starts. If the intervention arm is **inferior**, the trial is stopped, if it is **superior**, proof of concept, or recommendation for trial expansion can be declared.

Results: Recruitment has commenced and will be active at up to 15 sites.

Conclusion: This is the first registry-based multicentre trial in RMM to test the benefit of real-time PROM reporting. The widely used ePROM platform helps translation into practice and the pragmatic design suits rare diseases allowing a smaller sample to guide the decision to adopt real-time PROM reporting.

Factors associated with self-reported quality of life in a longitudinal study of patients with multiple myeloma (MM) in the Australian and New Zealand Myeloma and Related Diseases Registry (ANZ MRDR)

Moore E[1]
[1]*Monash University, Thornbury, Australia*

Aim: To identify factors associated with self-reported health-related quality of life (HRQOL) in patients with MM and change in HRQOL over time.

Method: Patients with a diagnosis of MM registered on the ANZ MRDR from Feb 2013 to Apr 2023 who had completed at least one EQ-5D-5L HRQOL survey were included. Surveys from different time points were analysed using the EQ5D utility score, a measure of HRQOL calculated from the 5 dimensions of the survey, using a generalised estimating equation. Utility scores range from '0' death to '1' full health; negative scores represent states considered worse than death. We investigated associations between the EQ5D utility score and demographic and clinical characteristics at diagnosis, and change in score in response to key 'events' such as autologous stem cell transplant. Pairwise comparison of values reported before and after autologous stem cell transplant (ASCT) was performed using the Wilcoxon signed-rank test.

Results: 2137 patients completed a total of 4796 surveys (median 2 [IQR1-4] per patient) with 1034 patients completing multiple surveys. The median time from diagnosis was 9 months (IQR 3-23). We found no association between utility score and age, gender, or ISS stage, however, poorer ECOG performance status at diagnosis was associated with poorer HRQOL (Table 1). Patients reported poorer HRQOL within three months of diagnosis than they did after this period. HRQOL (utility score) before an ASCT (0.71 [0.50-0.88] completed at median 5 [2-7] months before) was inferior to after (0.81 [0.66-0.93] completed at median 14m after [6-25]) ($p<0.001$).

Conclusion: HRQOL, measured by the EQ5D utility score, was associated with ECOG performance status at diagnosis, and improved with time from diagnosis. Having an ASCT was associated with improved HRQOL at median 14 m post ASCT. Further work is needed to investigate factors associated with the individual components of the EQ5D.

Table 1 – Results of multivariate GEE model of HRQOL

Factor	Coefficient (95% conf. interval)	P value
Age 60-65 vs < 60	-0.007 (-0.07,0.05)	0.81
Age 65-70 vs < 60	-0.003 (-0.06,0.05)	0.92
Age 70-80 vs < 60	-0.005 (-0.06,0.05)	0.84
Age > 80 vs < 60	-0.04(-0.11,0.03)	0.28
Gender – Female	-0.03 (-0.07,0.01)	0.15
ISS 2 vs 1	-0.02 (-0.07,0.03),	0.45
ISS 3 vs 1	-0.007 (-0.07,0.05)	0.81
ECOG 1 vs 0	-0.09 (-0.14,-0.05)	<0.001
ECOG 2 vs 0	-0.24 (-0.3,-0.18)	<0.001
ECOG 3 vs 0	-0.36 (-0.45,-0.3)	<0.001
ECOG 4 vs 0	-0.40 (-0.75,-0.06)	0.02
3-9 mths from Dx vs < 3 mths	0.09 (0.05,0.13)	<0.001
9-24 mths from Dx vs < 3 mths	0.11 (0.08,0.14)	<0.001
>24 mths from Dx vs < 3 mths	0.09 (0.05,0.12)	<0.001

Daratumumab Plus Lenalidomide and Dexamethasone (DRd) Versus Lenalidomide and Dexamethasone (Rd) in Transplant-Ineligible Patients with Newly Diagnosed Multiple Myeloma (NDMM): Clinical Assessment of Key Subgroups of the MAIA study

Quach H[1], Moreau P[2], Facon T[3], Usmani S[4], Bahlis N[5], Raje N[6], Plesner T[7], Orlowski R[8], Basu S[9], Nahi H[10], Hulin C[11], Goldschmidt H[12], O'Dwyer M[13], Aurore P[14], Venner C[15,16], Weisel K[17], Tiab M[18], Macro M[19], Frenzel L[20], Leleu X[21], Wang G[22], Pei H[23], Krevvata M[22], Carson R[22], Borgsten F[24], Shaji K[25]

[1]St Vincent's Hospital In Melbourne; Melbourne University, East Melbourne, Australia, [2]Hematology Department, University Hospital Hôtel-Dieu, Nantes, France, [3]University of Lille, CHU Lille, Service des Maladies du Sang, Lille, France , [4]Memorial Sloan Kettering Cancer Center, New York, USA , [5]Arnie Charbonneau Cancer Research Institute, University of Calgary, Calgary, Canada, [6]Center for Multiple Myeloma, Massachusetts General Hospital Cancer Center, Boston, USA, [7]Vejle Hospital and University of Southern Denmark, Vejle, Denmark, [8]Department of Lymphoma & Myeloma, The University of Texas MD Anderson Cancer Center, Houston , USA, [9]Royal Wolverhampton NHS Trust and University of Wolverhampton, CRN West Midlands, NIHR, Wolverhampton, UK, [10]Karolinska Institute, Department of Medicine, Division of Hematology, Karolinska University Hospital at Huddinge, Stockholm, Sweden , [11]Department of Hematology, Hôpital Haut Lévêque, University Hospital, Pessac, France, [12]GMMG-Study Group at University Hospital Heidelberg, Internal Medicine V, Heidelberg, Germany, [13]Department of Medicine/Haematology, NUI, Galway, Republic of Ireland, [14]CHU de Toulouse, IUCT-O, Université de Toulouse, UPS, Service d'Hématologie, Toulouse, France , [15]Department of Medical Oncology, Cross Cancer Institute, University of Alberta, Edmonton , Canada, [16]BC Cancer – Vancouver Centre Group, Vancouver, Canada, [17]Department of Oncology, Hematology and Bone Marrow Transplantation with Section of Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [18]CHD Vendée, La Roche sur Yon, France , [19]Centre Hospitalier Universitaire (CHU) de Caen, Caen, France, [20]Hôpital Necker-Enfants Malades, Paris, France , [21]CHU Poitiers, Hôpital la Milétrie, Poitiers, France , [22]Janssen Research & Development, LLC, Spring House , USA, [23]Janssen Research & Development, LLC, Titusville, USA, [24]Janssen Research & Development, LLC, Raritan, USA, [25]Department of Hematology, Mayo Clinic Rochester, Rochester, USA

Aim: The MAIA study evaluated DRd vs Rd in transplant ineligible NDMM. This analysis presents outcomes in clinically important patient subgroups such as age ≥75 years, International Staging System (ISS) stage III disease, renal insufficiency, extramedullary plasmacytomas at baseline, and high cytogenetic risk.

Method: In MAIA, 737 patients with NDMM ineligible autologous stem-cell transplant were randomised 1:1 to DRd or Rd. Patients were treated until disease progression or unacceptable toxicity. The primary endpoint was progression-free survival (PFS).

Results: Patient numbers in each treatment arm were similar across most subgroups (DRd; Rd): age ≥75 years (n=160; n=161); ISS stage III (n=107; n=110); renal insufficiency (n=162; n=142); extramedullary plasmacytomas (n=15; n=9); and high cytogenetic risk (n=48; n=44). After 64.5-months median follow-up, PFS (Fig.1A) and overall response rate (ORR; Fig.1B) generally favoured DRd versus Rd across subgroups. Minimal residual disease (MRD)-negativity rates were higher with DRd versus Rd for patients: aged ≥75 years (26.9% vs 9.9%; *P*<0.0001); with ISS stage III disease (27.1% vs 10.9%; *P*=0.0030); with renal insufficiency (29.6% vs 7.7%; *P*<0.0001); with extramedullary plasmacytomas (33.3% vs 0%; *P*=0.1181); and with high cytogenetic risk (25.0% vs 2.3%; *P*=0.0019).

Among patients aged ≥75 years, grade 3/4 treatment-emergent adverse events (TEAEs) occurred in 95.5% of DRd patients and 95.0% of Rd patients; the most common (≥20%; DRd/Rd) were neutropenia (62.4%/41.5%), lymphopenia (21.0%/12.6%), anaemia (20.4%/25.2%), and pneumonia (20.4%/14.5%). Serious TEAEs occurred in 80.9% and 79.2% of DRd and Rd patients, respectively.

Conclusion: In this subgroup analysis of MAIA, DRd improved PFS, ORR, and MRD-negativity rates versus Rd across clinically important subgroups. In patients aged ≥75 years, rates of grade 3/4 and serious TEAEs were similar with DRd and Rd. Results for these clinically important subgroups support the use of DRd as a standard of care for transplant-ineligible NDMM.

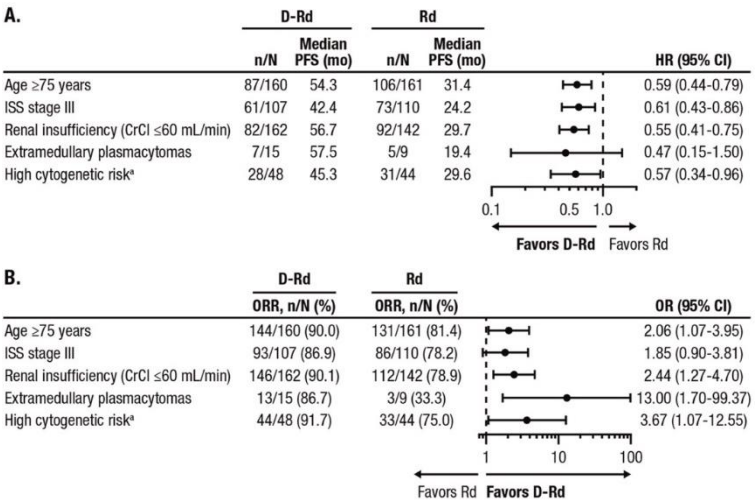


Figure 1. Subgroup analysis of PFS (A) and ORR (B) with DRd vs Rd.

PFS, progression-free survival; ORR, overall response rate; D-Rd, daratumumab plus lenalidomide/dexamethasone; Rd, lenalidomide/dexamethasone; HR, hazard ratio; CI, confidence interval; ISS, International Staging System; CrCl, creatinine clearance; OR, odds ratio.
[a]High cytogenetic risk was defined as having ≥1 of the following high-risk cytogenetic abnormalities: t(4;14), t(14;16), del17p.

Quality of life in patients with multiple myeloma treated with selinexor who had dose reductions: A subgroup analysis of the BOSTON study

Quach H[1,2], Jagannath S[3], Delimpasi S[4], Grosicki S[5], Špička I[6], Dimopoulos M[7]
[1]St Vincent's Hospital Melbourne, Fitzroy, Australia, [2]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne,, Australia, [3]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York,, USA, [4]General Hospital Evangelismos, Athens, Greece, [5]Medical University of Silesia, Katowice, Poland, [6]Charles University and General Hospital, Prague, Czech Republic, [7]National and Kapodistrian University of Athens School of Medicine, Athens, Greece

Aim: The oral XPO1 inhibitor selinexor is FDA-approved for relapsed/refractory multiple myeloma (RRMM) with dexamethasone (Xd), or bortezomib and dexamethasone (XVd) in adults with ≥1 prior therapy. Prescribing information recommends 100mg QW starting selinexor dose for XVd regimen, but median dose in BOSTON phase 3 trial (NCT03110562) was 80mg/week (range 30-137*). A previous analysis found selinexor dose reduction associated with reduced adverse events (AEs) burden and improved progression-free survival (Tables 1&2). Here, we assess the relationship between selinexor dose reduction and quality of life (QoL).

Method: BOSTON included 195 patients with MM randomized to selinexor QW (100mg), bortezomib QW (1.3mg/m^2) and dexamethasone BIW (20mg). QoL was assessed at baseline and day-1 of each cycle using EORTC QLQ-C30 (meaningful change threshold=10-point change).

Results: In total, 126 patients had selinexor dose reduction (median dose=71.3mg/week) and 69 did not (median=100mg/week; median age=66, median prior therapies=1 in both groups). Mean best change from baseline on EORTC QLQ-C30 was 10.0 (STD=20.5) in dose reduction group vs 4.0 (STD=20.9) in the group without (Table 3). In the dose reduction group, 54 patients (45%) achieved an increase of ≥10-points from baseline vs 20 patients (33%) in the group without. The first dose reduction was associated with a mean 4-point (STD=18.4) increase at next assessment and 12.8-point (STD=20.7) increase to best post-reduction score. The majority of the dose reduction group (66 patients [72.5%]) achieved their best post-baseline score (including tied for best) after first reduction.

Conclusion: Patients' QoL on average improved after selinexor dose reduction, consistent with our previous report that dose reduction was associated with reduced AE rates and improved tolerability and efficacy. These findings highlight the importance of dose reduction in optimizing RRMM treatment.

* >100mg/week possible due to dosing errors, shift in dosing schedule, or dose escalation after two cycles with no response.

Table 1. Efficacy outcomes after selinexor dose reduction

Group	N	PFS (mo) (95% CI)	ORR N (%)	sCR N (%)	CR N (%)	VGPR N (%)	PR N (%)	MR N (%)	SD N (%)
With selinexor dose reduction	126	16.6 (12.9, NE)	103 (81.7)	16 (12.7)	11 (8.7)	38 (30.2)	38 (30.2)	10 (7.9)	12 (9.5)
Without selinexor dose reduction	69	9.2 (6.8, 15.5)	46 (66.7)	3 (4.3)	3 (4.3)	16 (23.2)	24 (34.8)	6 (8.7)	13 (18.8)

Abbreviations: CI, confidence interval; CR, complete response; mo, months; MR, minimal response; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

Table 2. Duration-adjusted incidence of adverse events of clinical interest on or before first dose reduction after first dose reduction (safety population)*

Treatment-emergent adverse event	On or before first dose reduction in selinexor (N = 195)	After first dose reduction in selinexor (N = 126)
Thrombocytopenia	62.5	47.6
Neutropenia	10.6	7.7
Anemia	17.9	10.3
Nausea	31.6	7.3
Fatigue	28.1	9.9
Decreased appetite	21.5	6.4
Diarrhea	12.9	5.2
Weight decrease	9.0	5.9

* Duration-adjusted incidence of AE defined as the average number of events per 100 patients during a 4-week cycle.

Table 3. Quality of life status by selinexor dose reduction*

	All XVd Patients	
	With dose reduction in selinexor (N = 126)	Without dose reduction in selinexor (N = 69)
Patients with non-missing baseline and at least one post-baseline score, n (%)	121 (96.0)	61 (88.4)
Best change from baseline mean (STD) ≥ 10 pts, n (%)	10.0 (20.5) 54 (44.6)	4.0 (20.9) 20 (32.8)
Patients with at least one post-baseline score on or before first selinexor dose reduction, and at least one post-baseline score after first selinexor dose reduction, n (%)	91 (72.2)	
Change from last post-baseline score on or before first selinexor dose reduction to first post-baseline score after first selinexor dose reduction, mean (STD)	4.0 (18.4)	
Change from last post-baseline score on or before first selinexor dose reduction to best post-baseline score after first selinexor dose reduction mean (STD) ≥ 10 pts, n (%)	12.8 (20.7) 45 (49.5)	
Best post-baseline score achieved after first selinexor dose reduction (counting tied for best), n (%)	66 (72.5)	

* Quality of life scores assessed using the European Organization for Research and Treatment of Cancer 30-item core QoL questionnaire (EORTC QLQ-C30).

Carfilzomib Use among Patients with Relapsed/Refractory Multiple Myeloma in the Asia Pacific Region: Characteristics and Outcomes by Regimen from a Prospective, Real-World Study

Quach H[1], Yoon S[2], Kim K[3], Lee J[4], Nagarajan C[5], Ho P[6], Low M[7], Huang W[8], Wong R[9], Liao N[10], Lin C[10], Chai S[11], Britland M[12], Li J[11], Kim J[13]
[1]St.vincent's Hospital Melbourne, Fitzroy, Australia, [2]Seoul National University Hospital, Seoul, South Korea, [3]Department of Medicine, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, South Korea, [4]Dong-A University Hospital, Busan, South Korea, [5]SingHealth Duke-NUS Blood Cancer Center and Singapore General Hospital, , Singapore, [6]Royal Prince Alfred Hospital, Sydney, Australia, [7]Monash Medical Centre, Melbourne, Australia, [8]Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien City, Taiwan, [9]Prince of Wales Hospital, , Hong Kong, [10]Parexel International, Taipei, Taiwan, [11]Amgen Asia Holding Limited, , Hong Kong, [12]Amgen Australia Pty Limited, Sydney, Australia, [13]Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea

Aim: To describe utilization of carfilzomib (with lenalidomide-dexamethasone [KRd], with dexamethasone [Kd], or other regimens) for multiple myeloma (MM) in routine clinical practice in the Asia-Pacific, including treatment patterns, patient profile, clinical outcomes, and frequency of adverse events (AEs).

Method: This ongoing, prospective cohort study recruited adults with relapsed/refractory MM who received carfilzomib in routine clinical practice and ≥1 prior line of MM treatment. Medical history, patient characteristics, and clinical data are collected at baseline and throughout the 2-year observation period. AEs leading to treatment discontinuation are collected, common AEs associated with drugs/disease were excluded from collection by the protocol. All serious AEs were collected.

Results: As of 1-April-2022, 311 patients were enrolled, of whom 273 patients were included in this ad hoc interim analysis (Australia, n=46, Hong Kong n=17, Korea n=172, Singapore n=13, Taiwan n=25) (KRd 59%, Kd 38%, other regimens 4%). Overall, 33% of patients reported a history of hypertension, 11% cardiac disorders, 7% diabetes, and 3% pulmonary embolism. On average, KRd patients were younger and had received fewer lines of treatment than Kd patients (mostly 1-2 and 3-4+, respectively). At data cutoff, 43.1% KRd and 30.1% Kd patients remained on carfilzomib. The most common reason for carfilzomib discontinuation was disease progression/refractory disease. With KRd and Kd, respectively, the best overall response rate was 79.0% (n=94/119) and 54.7% (n=29/53) and median overall survival was 60.9 (95% confidence interval [CI], 55.9-not estimable [NE]) and 54.5 (95% CI, 33.3-NE) months. Overall, 10 (3.7%) patients discontinued carfilzomib owing to an AE.

Conclusion: Interim results of this prospective study in the Asia-Pacific region confirm that the standard dosing schedules for KRd and Kd are well tolerated in real-world practice and suggest a very low rate of discontinuation due to CFZ-related AEs, even in heavily pretreated MM.

Plasmablastic myeloma vs plasmablastic lymphoma, a diagnostic Dilemma

Ranjan A[1]
[1]*AIIMS, NEW DELHI, New Delhi, India*

Introduction: Plasmablastic myeloma (PM) and Plasmablastic lymphoma (PL) share clinical presentation have different therapeutic plans.

Method: Retrospective analysis of four cases.

Short clinical history: 1st case: A 31 year old immunocompetant male presented with oral ulceroproliferative growth. FNAC showed lymphoid cells with CD45+ve & HMB 45-ve. Biopsy showed lymphoplasmacytoid cells positive for CD79a, CD138, MUM1 & negative for CD20, CD3, CD56, CK & HMB45, suggesting PL. BMA smear & biopsy confirmed plasmablastic infiltration. CHOP therapy showed no improvement. Later raised serum free light chain ratio & B2M were found to change the diagnosis from PL to PM.
2nd case: A 57 year old female presented with swelling in alveolus. Punch biopsy showed plasmablasts, positive for CD 79a & CD138 and negative for CD 3, CD20, CD30 and CD 56. BMA and biopsy showed plasmacytoid cells positive for CD 138, negative for Pancytokeratin with proliferation index of 100% (MIB staining). Serum M- band was positive & B2M was raised. FISH showed negative del 17p, 13q, t(4,14), t(14,18) & IGH rearrangement. Considering PM, executed therapy showed poor response. Repeat HIV test turned positive to change the diagnosis.
3rd Case: A 34 year old HIV positive male, on HAART for six months presented with swelling in neck and right axilla. BMA showed plasmacytoid cell; CD38+, CD138+, kappa restriction, CD45+, CD20-, CD56-, CD19- and CD27dim. PL was considered.
4th case: An adult male with lymphadenopathy and positive CARB criteria had lymph node biopsy done showed sheets of plasmacytoid cells positive for CD 79a, MUM 1, CD 138, CD 38, CD 56, EMA and Cyclin D and negative for CD3, CD20, PAX 5, BCL6 &, CD10. Ki 67 proliferative index was 90%. **Light chian disease** was diagnosed.

Discussion: The distinction between PM and PL is still a challenge even at molecular level.

Imaging cardiac amyloidosis using 18F-florbetaben positron electron topography (PET) in systemic light chain (AL) amyloidosis.

Ranjit Anderson N[1,2], Tobin J[1,3], Abro E[1,2], Mollee P[1,2]
[1]Princess Alexandra Hospital, Woolloongabba, Australia, [2]University of Queensland, St Lucia, Australia, [3]Mater Research Institute, South Brisbane, Australia

Aim: 18F-florbetaben is a novel radiotracer that has previously demonstrated capability in delineating between cardiac amyloidosis and other causes of cardiac hypertrophy. We aim to assess the utility of 18F-florbetaben PET for cardiac imaging in patients with systemic AL amyloidosis.

Method: Newly diagnosed or previously treated patients with systemic AL amyloidosis and cardiac involvement were prospectively recruited to undergo a PET scan using 18F-florbetaben.

Percentage myocardial 18F-florbetaben retention (%MFBBR) was calculated from mean left ventricular (LV) myocardial standardised uptake values (SUV) at 0-5mins vs. 15-20mins. This was compared to current standard assessments of cardiac amyloidosis: LV global longitudinal strain (GLS), intraventricular septal wall thickness (IVs), serum brain natriuretic peptide (BNP), cardiac troponin I (cTnI) and cardiac staging (using Mayo 2004 staging for AL amyloidosis). Overall survival was calculated using Kaplan-Meir method.

Results: 17 patients (11 newly diagnosed, 6 previously treated) underwent a 18F-florbetaben PET scan between October 2013 and December 2020; 41% were female with a mean age of 62 years at diagnosis. The median follow up was 6.03 years; there were 8 deaths (47%), and median overall survival was 13.4 years.

High 18F-florbetaben uptake in the left ventricular wall was clearly demonstrated via visual assessment in all except 2 patients. When analysed, these 2 cases had a %MFBBR ≤ 40%, which has been previously been defined as a cut-off for excluding cardiac amyloidosis. On review, an alternative cause of heart failure or low likelihood of cardiac involvement with amyloidosis was found for these patients.

A correlation was demonstrated between %MFBBR and LV GLS (r=0.5, p=0.026). Participants with advanced cardiac disease (Mayo stage III), demonstrated higher SUVs (mean 9.26 vs 5.74) but this was limited by small numbers in this cohort.

Conclusion: 18F-florbetaben PET in AL amyloidosis is complementary to currently available assessment tools and may add value in assessing patients where cardiac involvement with amyloid is unclear and to identify patients with more severe cardiac amyloid burden.

A comparison of immunohistochemistry and laser microdissection tandem mass spectrometry to identify the amyloid fibril protein from formalin-fixed paraffin embedded biopsy samples.

Rowland J[1,2], Lambie D[1], Loo-Oey D[3], Cuthbertson K[1], Gibbs S[4,5,6], Hoskings P[4], Kwok F[7,8], Shingde M[9], Mollee P[1,2]

[1]Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia, [2]Queensland Amyloidosis Centre, Princess Alexandra Hospital, Brisbane, Australia, [3]Translational Research Institute, Brisbane, Australia, [4]Eastern Health, Monash University Clinical School, Box Hill, Australia, [5]Epworth Freemasons Hospital, Melbourne, Australia, [6]Alfred Health, Melbourne, Australia, [7]Westmead Amyloidosis Service, Westmead Hospital, Sydney, Australia, [8]Department of Clinical Haematology, Westmead Hospital, Sydney, Australia, [9]Department of Tissue Pathology, Westmead Hospital, Sydney, Australia

Aim: Correct diagnosis of amyloidosis subtype is a critical step to direct patient management, inform prognosis and guide targeted genetic testing. Laser capture microdissection and tandem mass spectrometry (LMD-MS) analysis of formalin-fixed paraffin-embedded (FFPE) biopsy samples is emerging as the new gold-standard diagnostic technique in amyloid subtyping, with likely superiority to conventional immunohistochemistry (IHC) based approaches.

Method: To assess this, a novel LMD-MS assay was developed. In brief, 10-micron sections were cut from FFPE biopsies, deparaffinised and stained with Congo red. Laser microdissection was performed of Congo red positive deposits. Proteins from dissected tissue were digested to peptides and analysed with high-performance liquid chromatography (HPLC) coupled with a ThermoFisher scientific Q Exactive plus mass spectrometer and peptide matches assessed against the Swiss-Prot/Uniprot human protein database with the addition of the Kabat library. 121 patient samples were assessed using both LMD-MS and an IHC panel consisting of 4 commercial antibodies: kappa, lambda, transthyretin and serum amyloid A. Each IHC was assessed independently by two experienced pathologists and graded quantitatively. LMD-MS was reported using institutional bioinformatic reporting algorithms.

Results: Biopsy sites were most frequently renal (25.6%), cardiac (17%) and gastrointestinal tract (25.6%). IHC assessment was considered non-diagnostic in 44% of samples. 121 samples were assessed for LMD-MS and 110 were samples were suitable for analysis with the assay . An amyloid subtype was confidently identified in 96% of samples analysed, with only 4 samples not identifying an amyloid forming protein. Concordance was assessed between LMD-MS and IHC. 3 cases of IHC typed light chain amyloidosis were reclassified as non-AL amyloid types and 3 cases of IHC typed AA amyloidosis were reclassified as AL-amyloidosis, both with potentially significant therapeutic implications.

Conclusion: Proteomic assessment of amyloid biopsy with laser capture microdissection and mass spectrometry is superior to immunohistochemical analysis using commercial antibodies for amyloidosis subtyping.

Multiple Myeloma remains the leading cause of death in New Zealand myeloma patients.

Shih S[1], Tiedemann R[2]
[1]Te Whatu Ora - Waitemata, Auckland, New Zealand, [2]Te Whatu Ora - Te Toka Tumai & University of Auckland, Auckland, New Zealand

Aim: To provide an update on survival outcomes in New Zealand patients with multiple myeloma (MM) and to determine the causes of mortality in these patients.

Method: Retrospective cohort analysis of the Myeloma and Related Disease Registry, New Zealand was conducted in two parts. The first part analyses overall survival in MM patients diagnosed between 2007 and 2020, stratified by 4-year intervals. Kaplan-Meier survival curve will be presented and linear regression employed to determine if overall survival has further improved within the last decade. Part 2 includes participants with newly diagnosed MM between 2007-2018. Their corresponding mortality data were extracted from NZ Mortality Collection database. Cause of death, stratified by years from diagnosis will be presented. Competing risk regression was employed to assess the multivariable survival data.

Results: Part 1: No statistically significant improvement in median overall survival was observed in those diagnosed between 2011-2014 versus 2015-2018. However, statistically significant improvement was observed in those diagnosed between 2007-2010 vs 2011-2014. Median overall survival was not reached for those diagnosed between 2019-2020 due to insufficient follow-up time.

Part 2: 72% of this cohort had died from MM-related causes of death. This does not improve even in those who have survived more than 5 years from the initial diagnosis. No significant difference in MM-related causes of death was found when stratified by diagnosis year.

Conclusion: MM-related cause of death remains the leading cause of death in New Zealand myeloma patients. The lack of improved overall survival between 2011 – 2018 is not unsurprising in the absence of new novel agents being state-funded. Findings reflect the ongoing unmet needs of patients with multiple myeloma in New Zealand.

POEMS syndrome masquerading as Guillain-Barre syndrome: a rare presentation of a rare disease

Walsh A[1], Mackay L[1], Wight J[1,2]
[1]*Queensland Health, Townsville, Australia,* [2]*James Cook University, Townsville, Australia*

POEMS (Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes) syndrome is a rare paraneoplastic multisystem disorder secondary to a plasma cell dyscrasia. The neuropathy is classically subacute to chronic in onset, frequently mistaken for chronic inflammatory demyelinating neuropathy (CIDP), with demyelination on nerve conduction studies. We report a case of POEMS syndrome presenting with a rapidly progressive sensorimotor polyneuropathy.

A 63 year old male presented with an acute ascending symmetrical sensorimotor polyneuropathy, requiring intubation for respiratory weakness within 5 days of symptom onset. He had no response to initial treatment with intravenous immunoglobulin and plasma exchange for a suspected acute inflammatory demyelinating polyneuropathy. Further investigations revealed a lambda monoclonal protein of 3g/L, lambda free-light-chain of 42mg/L and 10% plasmacytosis on bone marrow biopsy. Osteosclerotic rib lesions were present on imaging. Neurophysiology demonstrated severe mixed sensorimotor peripheral neuropathy, with active denervation and no recordable motor unit potentials on electromyography. Systemic manifestations of POEMS syndrome included bilateral papilledema, hypogonadotropic hypogonadism, plethoric skin changes and bilateral pleural effusions. His serum VEGF level was elevated (223ng/L). He was treated with 4 cycles of bortezomib, lenalidomide and dexamethasone induction, with subsequent clinical improvement to allow consolidation with a melphalan 200mg/m^2 autologous stem cell transplant. After 9 months, he remains in haematological partial remission (by IMWG myeloma response criteria), with good neurologic recovery, currently undergoing inpatient rehabilitation.

Rapidly ascending polyneuropathy as a presentation of POEMS syndrome has been previously described in the literature on only three occasions to our knowledge. This case demonstrates an atypical presentation of this rare disorder, and highlights the need for clinician consideration of POEMS syndrome as a differential diagnosis in any polyneuropathy with a co-existing lambda monoclonal gammopathy.

From positivity to negativity: CD138 antigenic shift post allograft in multiple myeloma

Wiltshire K[1], Morgan S[1], Bingham N[1]
[1]*Alfred Health, Melbourne, Australia*

Multiple myeloma (MM) is a bone marrow based, multifocal plasma cell (PC) neoplasm which is associated with production of either clonal immunoglobin fragments or light chain presence in serum and/or urine. Diagnostic workup routinely includes bone marrow aspirate and trephine (BMAT) looking at PC clonality, through immunohistochemistry or flow cytometry (FC), as well as, PC burden on trephine; often determined morphologically by CD138 immunohistochemistry (IHC).

Here we present a unique case of a 49yo female who displayed antigenic shift with downregulation of CD138 post therapy for her extramedullary relapse 13 months post non-myeloablative (NMA) allograft transplant for ultra-high risk non secretory MM.

At diagnosis 5CPCQ FC was performed on peripheral blood displaying a CD138+/CD38+/CD56+/CD19-/CytoIg- population. Management included 6 months of MM therapy and upfront NMA allograft with BMAT MRD monitoring with EuroFlow post transplant. Three months post allograft, patient was MRD positive with persistent CD138 expressing PC population. After six months post allograft she achieved MRD negativity; including on BMAT performed 13 months post allograft which was only days prior to relapse presentation with extramedullary plasmacytoma which was CD138 + on IHC and FC. She underwent therapy with daratumumab, carfilzomib, dexamethasone and cyclophosphamide; achieving a complete metabolic response after two cycles. A BMAT performed 6 months later for ongoing MRD monitoring showed new MRD positivity (0.091% of total nucleated cells) with antigenic shift, a loss of CD138 and upregulation of CD81; this population was also CD38+/CD56+/CD19-. This MRD population of CD138- PC has persisted since on subsequent BMAT FC testing. This unusual phenomenon resulted in 8-month delay in recognising a need to alter routine practice of ordering CD138 IHC on her trephine biopsies and switching to CD38 IHC testing.

This case highlights the importance of ongoing immunotypic review of clonal plasma cells in the multiply-relapsed, heavily treated, and particularly post allograft transplant MM patient population.

Plasmablastic myeloma: a rare and aggressive variant of plasma cell neoplasm

Wong C[1], Chen S[1]
[1]*Australian Clinical Labs, Clayton, Australia*

Objective: Plasmablastic myeloma (PBM), also described as anaplastic multiple myeloma, is a rare and highly aggressive form of plasma cell neoplasm that presents a diagnostic challenge due to its striking resemblance to plasmablastic lymphoma (PBL) in terms of anaplastic morphology and overlapping features of immunophenotype. It is crucial to accurately distinguish between these two entities because the treatment approaches differ significantly.

Methods: We conducted a comprehensive case survey encompassing the clinical presentation and investigative findings, followed by a literature review aimed at differentiating PBM from PBL.

Results: An 86-year-old male presented with constitutional symptoms and multiple myeloma defining events, including anaemia, hypercalcemia, acute renal impairment, and skeletal lytic lesions. HIV screening was negative. Twelve months prior, his protein electrophoresis revealed an IgG kappa paraprotein of 11g/L, which had increased to 25g/L on presentation. His blood film exhibited moderate rouleaux formation with occasional plasma cells and mild thrombocytopenia of 106x10^9/L. Bone marrow examination showed marked excess of plasma cells (71% on aspirate) with anaplastic morphology characterized by large multinucleated and blastic appearance. Immunohistochemistry in the trephine sample demonstrated 80-90% CD138+ plasma cells. Conventional karyotyping and FISH revealed a hypodiploid pattern, t(4;14), 1p-, 1q+, 17p-, and tp53. Unfortunately, the patient died shortly after diagnosis.

A systematic review including 163 cases of PBL and PBM revealed a similar immunophenotype characterized by CD38+,CD138+ and MUM1+. In contrast to PBL, PBM cases exhibit myeloma defining events, paraproteinaemia and are usually EBER- expression with no history of HIV. Additionally, PBM patients presented with myeloma-associated features such as paraproteinaemia or lytic lesions, whereas PBL cases showed EBER+ and HIV+ (39%) with lymphoma-associated findings such as lymphadenopathy[1].

Conclusion: Plasmablastic myeloma is a rare variant morphological form of plasma cell neoplasm that is associated with a dismal prognosis. An accurate diagnosis is critical for management.

References:

- Mori, H., Fukatsu, M., Ohkawara, H. et al. Heterogeneity in the diagnosis of plasmablastic lymphoma, plasmablastic myeloma, and plasmablastic neoplasm: a scoping review. Int J Hematol 114, 639–652 (2021). https://doi.org/10.1007/s12185-021-03211-w

Trend for improved survival with the addition of doxycycline as an anti-fibrillogenic agent in the treatment of systemic light chain (AL) amyloidosis: a single-center, retrospective analysis of 52 patients

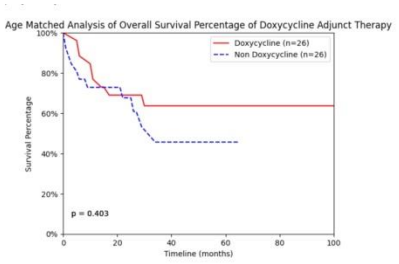
Wong C[1], Schwarer J[5], Tong A[4], Ting S[2,3], Gibbs S[2,3]
[1]Australian Clinical Labs, Clayton, Australia, [2]Eastern Health, Melbourne, Australia, [3]Australian Amyloidosis Network, Melbourne, Australia, [4]Monash University, Melbourne, Australia, [5]Alfred Health, Melbourne, 3000

Aim: Doxycycline is a broad-spectrum antibiotic used to treat bacterial infections. It has been shown *in vivo* and in mouse models to also inhibit amyloid fibrillogenesis (1). Retrospective American and British studies have suggested survival advantage for cardiac AL patients when doxycycline 100mg BD is added to their chemotherapy treatment (2,3) However, a recent prospective randomised Chinese study failed to confirm these findings with a bortezomib-based regimen.(4) We sought to examine our experience of the use of doxycycline as an anti-fibrillogenic agent in the treatment of AL patients in a retrospective analysis at a single Australian Amyloidosis service.

Method: A retrospective aged matched case control study of 52 patients diagnosed with AL patients treated between September 2014 and December 2020 at the Victorian and Tasmanian Amyloidosis Service at Eastern Health were identified, and their outcomes were analysed, based on whether adjuvant doxycycline was used or not in conjunction with systemic therapy.

Results: All patients were treated with at least one line of therapy with the majority receiving bortezomib, cyclophosphamide, and dexamethasone (VCD) upfront. 48 were of Caucasian background, 4 of Asian descent. Most patients were treated for 6 months or more. At the median follow-up of 25 months, survival analysis comparing patients who did and did not take doxycycline survival percentages of 69.02% and 67.67% (p=0.403), respectively (see graph).

Conclusion: Our analysis demonstrated no evidence of survival advantage when doxycycline was used with standard chemotherapy for the treatment of AL. The difference in outcomes reported between Caucasian and Chinese patients may be an area worth exploring for potential biological differences. Larger prospective studies would be of great interest.



References:

1. Ward JE, Ren R, Toraldo G, SooHoo P, Guan J, O'Hara C, et al. Doxycycline reduces fibril formation in a transgenic mouse model of AL amyloidosis. Blood. 2011; 118(25):6610-7.
2. Kumar SK, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Dingli D, et al. Doxycycline Used As Post Transplant Antibacterial Prophylaxis Improves Survival in Patients with Light Chain Amyloidosis Undergoing Autologous Stem Cell Transplantation. Blood. 2012;120(21):3138-.
3. Wechalekar AD and Whelan C. Encouraging impact of doxycycline on early mortality in cardiac light chain (AL) amyloidosis. Blood Cancer J 2017;7(3):e546.

A rare case of Ibrutinib causing Glanzmann's-like Platelet Defect in Advanced Waldenstrom's Macroglobulinemia

Aung W[1], **Baskaran A[1]**, Tong M[1], Zebelijan D[1], Hua M[1]
[1]*Liverpool Hospital , Sydney , Australia*

Brutons's tyrosine kinase inhibitor (Btk) Ibrutinib has proven efficacy in Waldenstrom's Macroglobulinemia (WM)[1]. Bleeding associated with its use is due to a combination of irreversible Btk inhibition and other off-target kinase inhibition[2].

We present an interesting case of a 72-year-old female with advanced multiply relapsed WM who developed an almost Glanzmann-like platelet defect on platelet LTA after 5 years on ibrutinib.

She was diagnosed with WM in 1994 and received multiple lines of immunochemotherapy over the two decades. Compassionate access to ibrutinib was granted in 2017 after an aggressive relapse necessitating plasma exchange. The dosing was reduced to 280mg due to drug-related fevers and liver function derangements. Her disease was well controlled on this reduced dose for five years before she started developing spontaneous bruising, petechiae, and epistaxis. Investigations at the time with platelet LTA demonstrated pan-inhibition of all agonists stimulated platelet responses showing in a Glanzmann-like pattern, as opposed to the reduction of collagen-induced platelet aggregation seen with ibrutinib therapy. She was switched to next-generation Btk inhibitor zanubrutinib with improved bleeding complications while maintaining disease control.

This case illustrates the therapeutic challenges in patients on ibrutinib and the potential acquired platelet dysfunction due to its off-target effects in keeping with the concept of dose and time-dependent platelet receptor shedding seen in ibrutinib and not in zanubrutinib[3]. This case supports the notion that next-generation BTK inhibitors have fewer off-target effects which translates to improved outcomes in patients on Btk inhibitors.

References

1. Steven P. Treon, M.D., Ph.D, Christina K. Tripsas, M.A., Kirsten Meid, M.P.H., Diane Warren, B.S., Gaurav Varma, M.S.P.H., Rebecca Green, B.S., Kimon V. Argyropoulos, M.D., Guang Yang, Ph.D., Yang Cao, M.D., Lian Xu, M.S., Christopher J. Patterson, M.S., Scott Rodig, M.D., Ph.D., et al, *Ibrutinib in Previously Treated Waldenström's Macroglobulinemia*.
2. Dmitrieva, E.A., et al., *Platelet function and bleeding in chronic lymphocytic leukemia and mantle cell lymphoma patients on ibrutinib.* Journal of Thrombosis and Haemostasis, 2020. **18**(10): p. 2672-2684.
3. Dobie, G., et al., *Ibrutinib, but not zanubrutinib, induces platelet receptor shedding of GPIb-IX-V complex and integrin αIIbβ3 in mice and humans.* Blood Advances, 2019. **3**(24): p. 4298-4311.

Congenital factor XIII deficiency: clinical presentation and molecular findings in a paediatric cohort

Flynn E[1], Furmedge J[1], **Barnes C[1]**
[1]Royal Children's Hospital, Melbourne, Australia

Background: Congenital factor XIII is rare yet severe bleeding disorder that affects approximately 1 in 2 million people[1,2]. Previously, Fibrogammin, a plasma-derived virally-inactivated product, has been used for both on-demand therapy and prophylaxis in factor XIII deficiency[1]. In recent years a recombinant product has become available in Australia and has been demonstrated to be a safe and effective option for prophylaxis in patients with A-subunit deficiency[1]. In anticipation of offering this option to patients receiving prophylaxis, patients with factor XIII deficiency at RCH were approached to offer molecular testing at their routine outpatient appointments. The samples were sent for next generation sequencing of the F13A1 and F13B genes. The clinical presentation of each of these cases was also reviewed.

Results: The cohort of patients with factor XIII deficiency at the Royal Children's Hospital included six patients aged between 17 months and 18 years. All patients were diagnosed in the first two weeks of life. Four patients were diagnosed due to umbilical bleeding between day 5 and 12, one patient had resultant hypovolemic shock and another required red cell transfusion. All patients had cessation of bleeding with either cryoprecipitate or factor XIII concentrate. The two remaining patients were younger siblings of known cases and diagnosed in the context of family history. Four of the patients received regular prophylaxis with plasma-derived FXIII (Fibrogammin). Four of the patients have had molecular testing and were homozygous for a pathogenic variant in the F13A1 gene.

Conclusion: The clinical presentations of this paediatric cohort highlight the importance of early consideration of factor XIII deficiency in infants presenting with umbilical stump bleeding. The recent availability of a recombinant factor XIII product warrants assessment for A-subunit deficiency in patients receiving prophylaxis, so that both products can be considered and discussed with patients and their families.

References:

- Inbal, A., Oldenburg, J., Carcao, M., Rosholm, A., Tehranchi, R., & Nugent, D. (2012). Recombinant factor XIII: a safe and novel treatment for congenital factor XIII deficiency. Blood, The Journal of the American Society of Hematology, 119(22), 5111-5117.
1. Bouttefroy, S., Meunier, S., Milien, V., Boucekine, M., Chamouni, P., Desprez, D., ... & CoDeC study group. (2020). Congenital factor XIII deficiency: comprehensive overview of the FranceCoag cohort. British Journal of Haematology, 188(2), 317-320.

Fulminant intravascular haemolysis as a consequence of Clostridium perfringens bacteraemia

Broadby G[1]
[1]*Royal Hobart Hospital, Hobart, Australia*

Introduction: *Clostridium perfringens* is a gram-positive, anaerobic bacillus found in intestinal tracts of humans[1]. *Clostridium perfringens* bacteraemia is a rare and life-threatening cause of intravascular haemolysis, mediated by the production of highly virulent alpha-toxin[1]. Early mortality is significant, with patients often dying within hours of presentation, and overall mortality has been shown to be 80%[2]. We present the case of a female patient with fulminant intravascular haemolysis secondary to *Clostridium perfringens* bacteraemia, as a complication of hysteroscopy with dilation and curettage (D&C) and in the context of gynaecologic malignancy.

Case Report: The patient was a 56-year-old female who underwent hysteroscopy with dilation and curettage (D&C) to investigate post-menopausal bleeding. She presented to hospital day 1 post-procedure acutely unwell with severe abdominal pain and was found to have a uterine perforation with associated haemorrhage and *Clostridium perfringens* bacteraemia. She underwent exploratory laparotomy, which showed uterine perforation but no bowel injury and haemostasis was achieved. She developed rapidly progressive haemolysis and multi-organ failure despite intensive care level support and appropriate antimicrobial therapy, and died the following day. Histopathology subsequently showed endometrial carcinoma with focal vascular invasion.

Conclusion: This case highlights the high early mortality associated with *Clostridium perfringens* bacteraemia associated haemolysis, and the need for prompt diagnosis and empirical management if suspected.

References:

- Kiu R, Hall LJ. An update on the human and animal enteric pathogen Clostridium perfringens. Emerging microbes & infections. 2018 Dec 1;7(1):1-5.
1. Van Bunderen CC, Bomers MK, Wesdorp E, Peerbooms P, Veenstra J. Clostridium perfringens septicaemia with massive intravascular haemolysis: a case report and review of the literature. Neth J Med. 2010 Sep 1;68(9):343-6.

Implementation of the Cepheid Xpert® Factor II & Factor V Assay for detection of Factor II (G20210A) and Factor V Leiden (G1691A) gene mutations in patients with suspected thrombophilia.

Caron D[1], De Silva W[1], Le A[1], Markalanda D[1], Agarwal R[1]
[1]*Austin Pathology, Blackburn North, Australia*

Aim: To validate the Xpert® Factor II & Factor V Assay for routine laboratory use in the detection of Factor II (FII) G20210A and Factor V Leiden (FVL) G1691A mutations in patients with suspected thrombophilia. The assay uses a closed system where DNA extraction, amplification and detection occur in one cartridge resulting in reduced labour costs and rapid turnaround times compared to in-house real-time PCR assays.

Method: A cost and clinical benefit analysis was undertaken to determine the suitability of the Xpert® Factor II & Factor V Assay for use in a Molecular Diagnostic Laboratory. Once deemed suitable, validation proceeded whereby 44 patient samples with known genetic status of FII and/or FVL gene mutations (12 FII, 18 FVL normal/wild type samples; 14 FII, 20 FVL heterozygous samples; and 3 FII, 2 FVL homozygous samples) were tested on the Cepheid GeneXpert Dx System. Results were assessed for concordance with the current in-house real-time PCR assay results.

Results: Validation of 44 samples showed 100% concordance with the in-house assay for both FII and FVL mutations and with 100% result reproducibility. There are cost-saving and clinical benefits implementing the Xpert® Factor II & Factor V Assay compared to the in-house assay due to the assay's simplicity and efficiency resulting in reduced labour (-90 minutes/week), training and turnaround times (bi-weekly instead of weekly).

Conclusion: The Xpert® Factor II & Factor V Assay is deemed cost-effective and clinically advantageous, and has been successfully validated for use. The simplicity and efficiency of this assay has resulted in faster turnaround times for clinicians to aid in diagnosis (and management) of patients with thrombophilia, and to free up staff for other laboratory work.

A retrospective audit of referrals for PLASMIC scores and ADAMTS13 testing – Does the PLASMIC score apply to real world patients?

Chandler A[1], Chapman K[1], Enjeti A[1]

[1]*Calvary Mater Newcastle, Waratah, Australia*

Aim:

- To review the characteristics of all referrals made for diagnostic ADAMTS13 testing over a two and half year period.
1. To determine whether PLASMIC scores are useful in predicting an ADAMTS13 level < 10% in the real world.

Method: An initial retrospective review of all referrals for ADAMTS13 testing where additional investigations were available for review was undertaken between 1/1/2020 to 1/9/2022. Reason for referral (if available), whether fragments were present on blood film, platelet count and parameters making up the PLASMIC score were analysed. An additional cohort of known ADAMTS13 deficient results (levels < 10%) from 2015 – 2020 was also reviewed and compared.

Results: A total of 342 referrals were screened in the study period. 186 referrals were included in the final analysis after follow up samples, relapsed TTP and incomplete data sets were removed. 62% of referred samples had fragments noted on the blood film. 18% of referred samples had a normal platelet count (> 150 x 10^9/L). 8 patients from the original cohort had an ADAMTS13 level < 10%. Of those patients who had an ADAMTS13 level < 10% (including an additional 4 patients) the PLASMIC scores ranged from 4 to 7. One patient had a PLASMIC score of 4 whilst all other patients had a PLASMIC score of 5 or greater.

Conclusion: Laboratory referrals for ADAMTS13 testing are different to the reference population from which the PLASMIC score was derived. A significant number of referrals in the real world are in patients who do not have fragmentation or thrombocytopenia. With the exception of one patient, all ADAMTS13 activity results of < 10% in the study correlated with a PLASMIC score of 5 or greater. The PLASMIC score can assist with decision making regarding testing for TTP, however this also needs to be considered within the clinical context and there may be occasional patients where the score may be low.

ENERGY trial in warm autoimmune hemolytic anemia (wAIHA): design of a phase 2/3 randomized, double-blind, placebo-controlled study to assess the efficacy and safety of nipocalimab, an FcRn blocker

Choi P[1], Murakhovskaya I[2], Fattizzo B[3], Ebrahim T[4], Sweet K[4], Shu C[5]
[1]The Canberra Hospital, Hematology Clinic/National Platelet Research and Referral Centre, The Australian National University, Canberra, Australia, Garran, Australia, [2]Albert Einstein College of Medicine/Montefiore Medical Center, Department of Hematology and Oncology, Bronx, USA, [3]Fondazione IRCCS Ca'Granda Ospedale Maggiore Plicilinico, Hematology Unit, Milan, Italy, [4]Janssen Pharmaceutical Companies of Johnson & Johnson, Cambridge, USA, [5]Janssen Research & Development, LLC, Spring House, USA

Aim: Warm autoimmune hemolytic anemia (wAIHA) is characterized by the premature destruction of red blood cells mainly in the presence of pathogenic immunoglobulin G (IgG) autoantibodies. Nipocalimab targets the neonatal Fc receptor (FcRn) to lower circulating IgG levels, including pathogenic autoantibodies. We describe the rationale and study design of ENERGY, an ongoing, adaptive, phase 2/3 multicenter, randomized, double-blind, placebo-controlled study evaluating efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of nipocalimab compared with placebo in pts with wAIHA (NCT04119050).

Methods: Pts ≥18 years of age who have been diagnosed with primary/secondary wAIHA and are currently receiving treatment for wAIHA/have previously received treatment for wAIHA will be included in the study. Pts with cold antibody AIHA, cold agglutinin syndrome, mixed type (warm and cold) AIHA, or paroxysmal cold hemoglobinuria will be excluded. Stable doses of corticosteroids or immunosuppressants will be allowed. Approximately 111 pts will be randomized 1:1:1 to receive nipocalimab at 2 different dose schedules or placebo. Following completion of 24 weeks of double-blind treatment, pts may enter an open-label extension period to receive nipocalimab for 144 weeks with a follow-up period of 6 weeks after last assessment.

Results: ENERGY will include approximately 160 sites across 19 countries, including Brazil, Canada, China, Czech, Egypt, France, Germany, Greece, Hungary, Japan, Israel, Malaysia, Netherlands, Italy, Poland, South Korea, Spain, the United Kingdom, and the United States. The primary endpoint is percentage of pts achieving durable response of improvement in hemoglobin. Secondary endpoints include change from baseline in the total score from the Functional Assessment of Chronic Illness Therapy-Fatigue Scale, corticosteroid dose reduction from baseline, and normalization of hemolytic markers.

Conclusion: The results of ENERGY have the potential to identify a novel treatment option to address the significant unmet needs of pts with wAIHA. Enrollment is ongoing in this clinical trial.

Microangiopathic haemolytic anemia in a patient with severe COVID-19 infection: a case report.

Droney R[1], Wilson C[1]
[1]*Mater Hospital, South Brisbane, Australia*

Microangiopathic haemolytic anemia in a patient with severe COVID-19 infection: a case report. A 76-year-old female with dyspnoea, diarrhoea and hypoxia ($Sa0_2$ of 92% on room air) was diagnosed with moderate-severe COVID-19. She was treated with Remdesivir and Dexamethasone. On day 2 of her admission, she became delirious and developed further hypoxia and so was intubated in the intensive care unit. A microangiopathic haemolytic anaemia (MAHA) developed with thrombocytopenia ($32x10^9$/L), anaemia (haemoglobin 81g/L), red cell fragmentation, a low haptoglobin <0.2g/L and a raised LDH (1266U/L). An acute kidney injury was also identified with a creatinine of 169umol/L. A medication review did not reveal any medications associated with a MAHA. Urgent plasma exchange was commenced with an improvement in haematological parameters and her sensorium. Subsequently the ADAMTS13 was 0.67U/mL and the Complement factor B, complement factor I and complement factor H were all normal. The Shiga-like toxin was not detected in the faeces. She was given corticosteroids, and plasma exchange was undertaken daily for 6 days which resulted in a significant clinical benefit with normalisation of the haematological parameters, haemolysis and renal function. There have been several case reports of a MAHA being associated with COVID-19 infections. Some of these cases have been confounded by other potential causes of MAHA. It is thought that COVID-19 can trigger thrombotic thrombocytopenic purpura, or unmask or an underlying complement defect and trigger an atypical haemolytic uraemic syndrome. Various treatments have been used for patients with MAHA associated with COVID. Benefit has been reported in cases treated with corticosteroids and plasma exchange, much like our patient discussed here. Clinicians should be alert to this potentially serious complication of COVID-19, to ensure prompt management leads to the best possible patient outcomes. Further study is required to determine the precise pathophysiological mechanism of MAHA in COVID-19, and the optimal treatment strategy for these patients.

Lupus anticoagulant hypoprothrombinaemia after a viral illness, resulting in haematuria: a case report.

George H[1], Brown S[2], Don M[3]
[1]Pathology Queensland, Royal Brisbane And Women's Hospital, Brisbane, Australia, [2]Queensland Children's Hosptial , Brisbane, Australia, [3]St Vincent's Private Hospital, Toowoomba, Australia

Lupus anti-coagulant hypoprothrombinaemia syndrome (LAHPS) is an infrequently encountered, rare cause of bleeding and can be challenging to clinically manage. Bleeding occurs due to acquired antibodies against coagulation factor II (FII) and treatment can vary depending on the cause of the lupus anti-coagulant.

We present the case of a four-year-old male, who presented with haematuria one week after a diarrhoeal illness. He had no associated urinary tract symptoms or other infective symptoms. Laboratory tests demonstrated a normal full blood count, including a normal platelet count, with an abnormal coagulation profile. His prothrombin time (PT) was prolonged at 34 seconds, Echis time was greater than 60 seconds and activated partial thromboplastin time (aPTT) was prolonged at 101 seconds. Further testing demonstrated a positive lupus anticoagulant and a coagulation factor II (FII) level of less than 1%.

The patient had no symptoms concerning for an underlying auto-immune disorder and the rest of the auto-immune panel was negative. He had no previous personal or family history concerning for a bleeding disorder. He was diagnosed with LAHPS because of a transient, virally induced lupus anti-coagulant. Given his clinical bleeding, he was treated with three days of intravenous methylprednisolone (10mg/kg) and the repeat FII level increased to 5% by the end of treatment. The patient has progressed favourably since then and a repeat coagulation profile approximately 3 weeks post treatment, demonstrated a PT of 11 seconds and an aPTT of 48 seconds, with a FII level of 91%.

This case highlights the need for clinicians to consider LAHPS as a cause of bleeding in children, especially those with a recent infection and adds to the body of literature supporting corticosteroids as a possible treatment strategy.

Investigating the effectiveness of bevacizumab in patients with Hereditary Haemorrhagic Telangiectasia at Gold Coast Health

Georgiadis N[1]
[1]*Gold Coast Hospital and Health Service, Southport, Australia*

Background: Bevacizumab is a monoclonal antibody that inhibits vascular endothelial growth factor. It is currently used off label in many centres worldwide to help improve patients symptoms in hereditary hemorrhagic telangiectasia (HHT).

Aim: The aim was to investigate at whether patients with HHT had an improvement in their symptoms after receiving bevacizumab.

Method: The number of red cells, iron infusion and epistaxis severity scores before and after receiving bevacizumab were compared in two patients at Gold Coast University Hospital.

Results: The number of iron infusions, red cells and epistaxis severity score decreased after the patients received bevacizumab.

Conclusion: This adds to the growing evidence that bevacizumab is an effective drug in helping to decrease the severity of iron deficiency anaemia and epistaxis in patients with HHT.

Evaluation of a Digital Morphology Analyzer for Improving Work Efficiency in a Private Referral Laboratory

Han Y[1], Hong S[1], Lee S[1], Lee Y[1], Lee S[1]
[1]*Seegene Medical Foundation, Seoul, South Korea*

Aim: Recently, digital morphology analyzers have been introduced to many institutions to help with laboratory work. Our institution, as a private referral laboratory, had an opportunity to use a peripheral blood analyzer (Sysmex DI-60; Sysmex, Kobe, Japan) and wanted to evaluate the performances of the equipment.

Method: From July to August 2022, a short term imprecision test of the equipment (one sample was read twice by the equipment, the standard deviation (SD) of the result was calculated and the average is obtained for each item) and a classification agreement test between the equipment and the microscope were performed with 22 samples.

Results: In the case of the imprecision test, the SD values were 2.14, 2.17, 2.61, 0.91, and 0.98 in the order of seg, lym, mono, eos, and baso, respectively. In the classification agreement test, the total number of classified cells was 5022, and the number of cells read with the same type on the equipment and microscope was 4462, indicating an agreement rate of about 88.8%. The proportion of devices classified as unidentified was approximately 2.8%.

Conclusion: Di-60 showed a high concordance rate with reading through direct microscope, and is considered to be a device that can help improve laboratory work efficiency in haematology laboratories, but accurate cell identification was not achieved in about 12%. Considering these points, the digital morphology analyzer should still be used as an auxiliary tool in the laboratory. In particular, in the case of patients with hematologic disease such as acute leukemia or related diseases, it is necessary to carefully examine whether there is a difference between the result of the equipment and the result of the microscope.

Clinical effectiveness, treatment use, satisfaction and impact on quality-of-life of pegcetacoplan treatment for patients with paroxysmal nocturnal haemoglobinuria (PNH).

Hsu D[1], Wilson K, Rich C, Hakimi Z, Horneff R, Fishman J, Mellor J, Earl L, Taylor Y, Simons A, Conyers J, Roeth A
[1]*Haematology Department, Liverpool Hospital, Liverpool, Australia*

Aim: Examine treatment use, satisfaction, clinical effectiveness, and impact on patient Quality of Life (QoL) of pegcetacoplan treated patients with PNH.

Method: Data were drawn from a a cross-sectional survey of hematologists and their consulting pegecetacoplan-treated PNH patients from January-November 2022 in the US, France, Italy, Germany, and Spain. Physicians reported patient demographics, clinical markers, treatment satisfaction, and QoL; patients self-reported treatment satisfaction and QoL. Descriptive statistics are shown.

Results: Sixty one patients were recruited. Mean (SD) time since PNH diagnosis: 3.7 (3.3) years; mean (SD) time receiving pegcetacoplan: 5.9 (4.0) months; and 57 (93.4%) previously switched from a C5 inhibitor (C5i). Physicians reported a mean (SD) improvement in haemoglobin of 2.5 (1.9) g/dL, 2.8 (2.1) g/dL, and 3.3 (2.1) g/dL for those receiving pegcetacoplan for ≥1 month (n=61), ≥3 months (n=44), and ≥6 months (n=23), respectively.

From initiation to data collection, lactate dehydrogenase improvement (<1.5 x ULN) was seen in 30.0% vs 57.4%, and no fatigue was reported in 1.6% vs 31.1% of patients respectively.

Physicians considered all patients to have ‘well or very well controlled’ disease and were ‘satisfied or completely satisfied’ with pegcetacoplan. Physicians reported 57.4% (n=35) of patients had ‘excellent or very good’ QoL.

For 91.1% (n=51/56) of patients switching from C5i to pegcetacoplan, physicians reported higher satisfaction with pegcetacoplan, mostly due to improved disease control (n=47/51, 92.2%). 30 patients provided self-reported data via questionnaire; 96.7% (n=29) were ‘satisfied or completely satisfied’ with pegcetacoplan and 92.9% (n=26) reported higher satisfaction vs previous C5i, with 76.9% (n=20) attributing higher satisfaction to experiencing less fatigue.

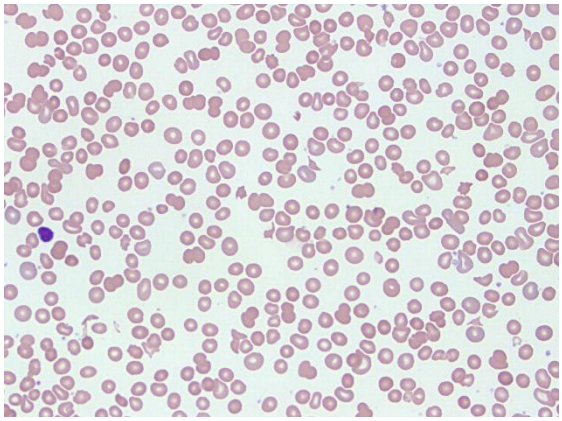
Conclusion: These findings demonstrate the real-world effectiveness of pegcetacoplan through improvement in clinical markers, and patient reported outcomes Further research is required to support the real-world benefits of pegecetacoplan treatment for PNH.

Haemolytic anaemia following robotic assisted mitral valve repair

Lee V[1], Wong W[2], Thomas L[2,3,4], Curnow J[1,3]
[1]Department of Haematology, Westmead Hospital, Westmead, Australia, [2]Department of Cardiology, Westmead Hospital, Westmead, Australia, [3]University of Sydney, Camperdown, Australia, [4]University of New South Wales, Kensington, Australia

Introduction: Haemolytic anaemia following mitral valve (MV) repair, rather than replacement, is a rare phenomenon. The authors present a novel case following robotically assisted surgery.

Case presentation: A 71-year-old man presented with symptomatic anaemia 12 months after robotic MV repair for posterior leaflet prolapse. Examination was significant for conjunctival pallor and a pan-systolic murmur. Investigations demonstrated a normocytic normochromic anaemia with iron deficiency, raised LDH, reticulocytosis, undetectable haptoglobin and raised creatinine. Blood film demonstrated polychromasia with8 fragments per HPF and a normal platelet count (figure 1). DAT, ADAMTS-13 (49%), PNH markers, autoimmune panel, APS and infective serology were negative.



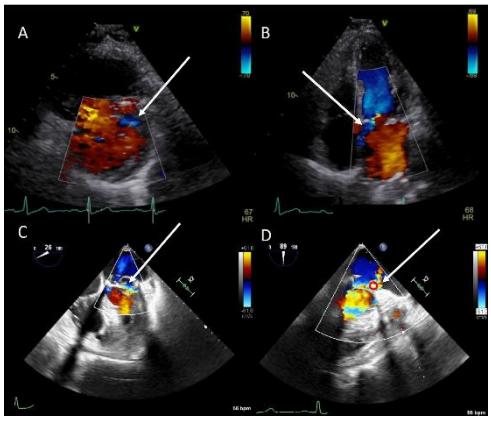


Figure 1 – Red cell anisocytosis, polychromasia and schistocytes at 40x magnification.

Figure 2 – TTE (A-B) and TOE (C-D). MR (white arrows). Cross section of the Cosgrove Band (red circle). MR flow is seen hitting the annuloplasty ring and flowing around it.

Robotic MV repair consisted of sub-valvular neo-chordae reconstruction to the prolapsing P2-segment and mitral annuloplasty with a Cosgrove band. On presentation, transthoracic echocardiogram (TTE) demonstrated mild-moderate anteriorly directed mitral regurgitation (MR) (figures 2A-B). Non-mechanical MV haemolysis in the absence of significant valvular or paravalvular regurgitation or significant stenosis was thought unlikely by the Cardiologist. However, further invasive anaemia workup of bone marrow, renal and colonoscopy biopsies was unremarkable, thus transoesophageal echocardiogram (TOE) was performed. This showed moderate prolapse of the posterior mitral leaflet with moderate eccentric MR directed anteriorly at the annuloplasty ring (figures 2C-D), likely causing haemolysis. Re-do surgery revealed new ruptured chordae lateral to the previous neo-chordae implantation causing MR. Laboratory markers subsequently normalised.

Discussion: Increased awareness of valve-related haemolysis after robotic MV repair is warranted as surgical treatment rapidly resolves the clinical problem. Diagnostically, TOE was superior to TTE in visualising the eccentric MR jet and its ‘collision’ with the prosthetic ring.

Conclusion: MV repair with mild-moderate MR on TTE should not be overlooked as a potential cause of haemolysis.

Diagnosis, antenatal and postnatal management of coexisting alpha thalassaemia, PK deficiency, and G6PD deficiency

Lindell-Innes R[1], Emmett S[1]
[1]*The Gold Coast University Hospital , Gold Coast, Australia*

Aim: We describe a case study of a fetus with concurrent heterozygous SEA alpha thalassaemia, G6PD, and PK deficiency, with a discussion of the literature.

Case: A healthy mother with known SEA alpha thalassaemia heterozygosity presented to routine antenatal clinic and was found to have a fetus with progressive ascites, with normal structural imaging and negative maternal antibody screening. Subsequent mean cerebral artery peak systolic velocities suggested discordant anaemia for alpha thalassaemia trait, and an intrauterine transfusion with chorionic villous sampling for DNA analysis was performed. Fetal whole exome sequencing (WES) by NGS identified an inherited heterozygous SEA deletion, though unexpectedly pyruvate kinase (PK) deficiency, as well as glucose-6-phosphatase deficiency (G6PD), manifesting as a microcytic haemolytic fetal anaemia. Partner testing was performed and careful management with 3 additional CMV negative, irradiated, fetal genotype matched intrauterine transfusions resulted in a live birth.

Discussion: Alpha thalassaemia, G6PD and PK deficiency each individually are uncommonly but reliably encountered in antenatal haematology. Haemoglobinopathies and metabolic red cell disorders coinheritance has been described; a cohort study of 79 Nigerian children in 2021 described a relationship between alpha thalassaemia or G6PD with sickle cell anaemia with a presumed protective effect. However, to the Author's knowledge, simultaneous alpha thalassaemia trait, PK and G6PD coinheritance is currently unreported in the literature. The case demonstrates lessons in diagnosis, counselling, and antenatal management. Further considerations for the potential protective effect of coinheritance, and the long term management of the newborn deserve consideration.

Conclusion: Coinheritance of alpha thalassaemia trait, PK and G6PD is, at the time of submission, unreported in the literature, though can be supported to live birth with careful antenatal management. Lessons in diagnosis and management may assist future similar cases.

Pelvic sepsis following fertility preservation procedures in severe aplastic anaemia

Maher-Pyrke D[1], Cashion C[2], Greenwood M[2], Johnston A[1]
[1]*Royal Hobart Hospital, Hobart, Australia,* [2]*Royal North Shore Hospital, Sydney, Australia*

Aim: Allogeneic haematopoietic stem cell transplant (SCT) is the standard of care in young patients with severe aplastic anaemia (SAA), where a suitable donor is available. In female patients, conditioning for allogeneic SCT may result in premature ovarian failure and infertility and so fertility preservation with oocyte retrieval may be considered prior to transplantation. These patients are likely at increased risk of complications from fertility preservation procedures due to neutropaenia and thrombocytopaenia, however, there is limited literature regarding the safety of fertility preservation in this population. Our aim is to highlight the potential risks.

Method: We present the cases of two young female patients with SAA managed at two different centres, who both experienced major complications and morbidity following fertility preservation procedures prior to planned allogeneic SCT.

Results: Patient 1 was a 21-year-old female who developed a large pelvic haematoma immediately following oocyte retrieval and subsequently developed polymicrobial bacteraemia due to secondary infection of the haematoma. Patient 2 was a 29-year-old female who was admitted with neutropaenic sepsis 9 days after oocyte retrieval and was found to have *Enterobacter cloacae* bacteraemia and a large pelvic collection. Both patients had prolonged, complicated hospital admissions, required surgical drainage of their pelvic collections, and received granulocyte transfusion support. Both patients were treated with antithymocyte globulin (ATG), cyclosporine and eltrombopag for their SAA without response. Patient 1 proceeded with haploidentical allogeneic SCT while patient 2 is planned for a second course of ATG.

Conclusion: These two cases highlight the potential risks of fertility preservation procedures in patients with severe aplastic anaemia, adding to the limited body of literature in this setting.

Case Study: Alectinib-induced direct antiglobulin test negative haemolytic anaemia

Niranjan N[1], Sanmugarajah J[1], Pelly T[1]
[1]*Gold Coast University Hospital, Southport, Australia*

Background: Alectinib is an oral tyrosine kinase inhibitor that selectively inhibits anaplastic lymphoma kinase (ALK).[1] It is utilised in treatment of ALK rearranged metastatic non-small cell lung cancer (NSCLC).[1, 2] Red cell morphological changes, reticulocytosis and reduced eosin-5-maleimide (e5m) binding is near universal in patients treated with alectinib.[3-5] Emerging research has shown a subset of patients develop clinically significant direct antiglobulin test (DAT) negative haemolytic anaemia which is reversible on drug cessation..[5, 6]

Case Study : A 62-year-old female with metastatic ALK rearranged NSCLC had a normal haemoglobin with no biochemical evidence of haemolysis at baseline. Within 1 month of alectinib therapy, she developed a mild hyperbilirubinemia and within 2 months she was anaemic. As the abnormalities were initially very mild, she continued alectinib, and haemolysis testing was not performed. She had been on alectinib for 12 months with excellent disease control when her haemoglobin dropped below 100g/L. A blood film was then reviewed (Figure 1), revealing marked red cell anisopoikilocytosis and polychromasia which is characteristic of alectinib-induced haemolysis.[3-7] A haemolytic screen was performed, revealing a DAT negative haemolytic anaemia (Table 1) and reduced e5M staining again consistent with alectinib-induced haemolysis.[3] Alectinib was thus permanently discontinued, and the patients' haematological parameters returned to normal within 2 months of drug cessation. She was subsequently commenced on brigatinib, an alternative ALK inhibitor, with no recurrence of haematological toxicity.

Conflict of Interests Statement
No conflicts of interest to disclose

References
1. Okumoto J, Sakamoto S, Masuda T, et al. Alectinib-induced immune hemolytic anemia in a patient with lung adenocarcinoma. *Intern Med*. 2021;60(4):611–615. doi:10.2169/internalmedicine.4241-19.
2. Kunz J, Wiedemann C, Grosch H, et al. Early development of ubiquitous acanthocytosis and extravascular hemolysis in lung cancer patients receiving alectinib. *Cancers*. 2022;14(11):2720. doi:10.3390/cancers14112720.
3. Kuzich JA, Heynemann S, Geoghegan N, et al. Alectinib induces marked red cell spheroacanthocytosis in a near-ubiquitous fashion and is associated with reduced eosin-5-maleimide binding. *Pathology*. 2021;53(5):608–612. doi:10.1016/j.pathol.2020.10.023.
4. Dores GM, Nayernama A, Cheng C, Moureaud C, Jones SC. Hemolytic anemia following alectinib reported to the U.S. food and drug administration adverse event reporting system. *Am J Hematol*. 2022;97(4):E129–E132. doi:10.1002/ajh.26454.
5. Yuan Y, Mapp S, Xu W. Two cases of marked red cell anisopoikilocytosis and haemolysis with alectinib, an anaplastic lymphoma kinase inhibitor. *Br J Haematol*. 2020;190(5):642. doi:10.1111/bjh.16813.
6. Gullapalli V, Xu W, Lewis CR, Anazodo A, Gerber GK. A multi-centre case series of alectinib-related erythrocyte membrane changes and associated haemolysis. *J Hematopathol*. 2021;14(2):131–136. doi:10.1007/s12308-020-00427-3.
7. Legland ÉP, Dejean AM, Plantamura J, et al. Acanthocytosis in an alectinib-treated patient. *Br J Haematol*. 2022;197(2):131. doi:10.1111/bjh.18020.

Table 1: *Patient's longitudinal haematological parameters*

	Prior to alectinib	6 months after commencement	12 months after commencement	1 month post cessation	2 months post cessation
Haemoglobin(g/L)	135	102	98	122	134
Bilirubin (umol/L)	10	29	35	11	7
Reticulocyte count ($x10^9$/L)	-	-	158	60	-
Haptoglobin (g/L)	-	-	< 0.01	0.48	-

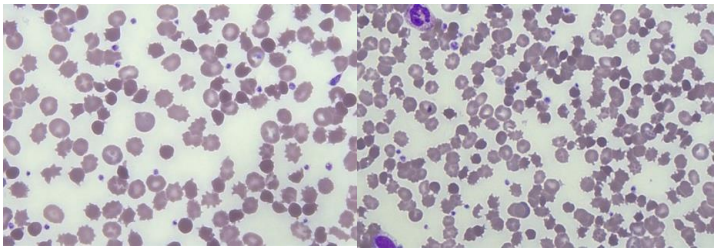


Figure 1: *Peripheral blood film stained with marked red cell anisopoikilocytosis (acanthocytes, echinocytes and spherocytes) and polychromasia.*

Haematopoietic effects of nitrous oxide: an unusual differential of granulocytic vacuolation

Noye J[1], Klose N[1], Harvey Y[1]
[1]*Sullivan Nicolaides Pathology, Brisbane, Australia*

Title: Haematopoietic effects of nitrous oxide: an unusual differential of granulocytic vacuolation Nitrous oxide is rising as a drug of abuse in Australia, with increasing incidence of hospital presentations over the last decade. While the most significant adverse effects are neurological, haematologic toxicities are also reported.

We present a case of a 40-year-old female who presented with paraparesis and dorsal column signs in the context of chronic nitrous oxide use – approximately one cannister second-daily for the preceding three months. Haematological parameters were: Hb 109g/L, MCV 99fL, WCC 3.4x10^9/L, ANC 0.27x10^9/L, and plt 189x10^9/L. Blood film showed mild left shift, macropolycytes, oval macrocytes, and hypersegmented neutrophils. Active B12 (>128pmol/L) and folate (38nmol/L) levels were normal. Lead, copper, and zinc levels were normal. Ancillary investigations showed elevated homocysteine (129.4umol/L, ref: <15umol/L) and methylmalonic acid (6.94umol/L, ref: <0.5umol/L), which are consistent with nitrous oxide toxicity. MRI demonstrated signal alteration through the cervical cord dorsal columns, consistent with subacute combined degeneration of the cord. She proceeded to bone marrow aspirate/trephine, which showed evidence of megaloblastosis, vacuolation within the granulocytic series, and occasional macropolycytes (Figure 1). She was treated with hydroxycobalamin 1mg intramuscularlydaily for her neurological manifestations and enoxaparin 40mg subcutaneouslydaily for venous thromboembolism prophylaxis. Haematologic parameters normalised over the following ten days.

Transient haematologic toxicity of nitrous oxide was noted in the 1980s, primarily mediated through irreversible inactivation of vitamin B12. Characteristic findings on bone marrow examination include megaloblastosis, as expected given this mechanism. Most literature has focused on short exposure durations during anaesthesia. Granulocytic vacuolation was an interesting feature in this case, which has been occasionally reported in addition to giant and binucleate granulocytic precursors in cases of prolonged exposure [1]. Given the rising incidence, nitrous oxide toxicity is an important differential of cytopenias to consider in the appropriate context. Biochemical findings with increased homocysteine and methylmalonic acid are characteristic.

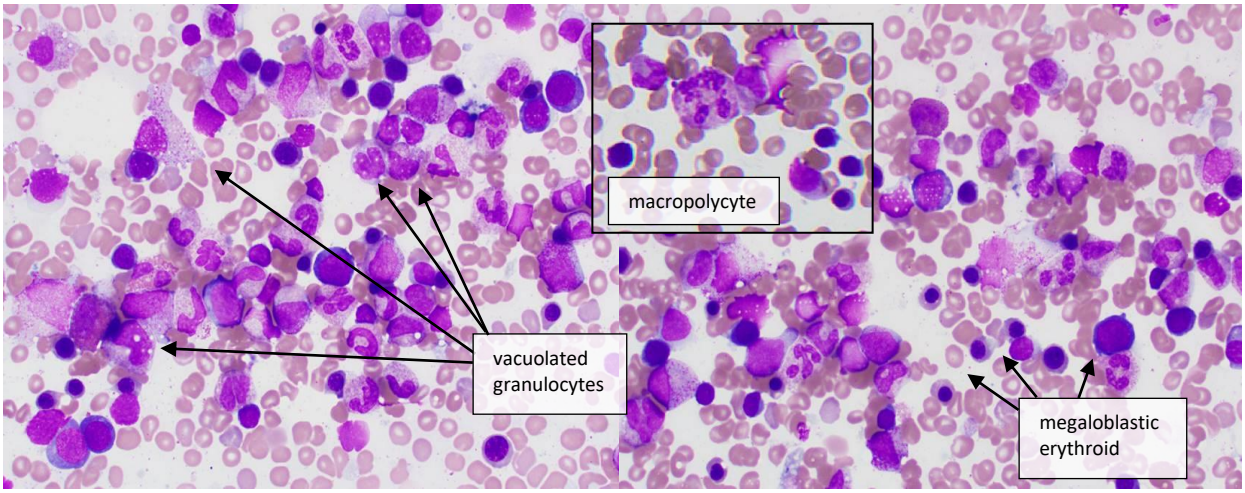


Figure 1. Bone marrow morphology in this patient.

1. Skacel PO, Hewlett AM, Lewis JD, Lumb M, Nunn JF, Chanarin I. Studies on the haemopoietic toxicity of nitrous oxide in man. Br J Haematol. 1983 Feb;53(2):189-200. doi: 10.1111/j.1365-2141.1983.tb02011.x. PMID: 6821648.

Acute pancreatitis causing microangiopathic haemolytic anaemia and thrombocytopenia

Peters L[1], Lai H[1]

[1]*Townsville University Hospital, Townsville, Australia*

Thrombotic microangiopathy (TMA) is characterised by the triad of microangiopathic haemolytic anaemia (MAHA), thrombocytopenia and end organ dysfunction. Prompt recognition and early intervention are essential to improve outcomes. TMA syndromes can be either primary or secondary to many systemic disorders. Here we present a case of acute pancreatitis associated TMA which is rarely reported in literature.

A 39-year-old female presented with clinicoradiological acute, calculous, necrotising pancreatitis with a lipase of 3000U/L. Full blood count on admission demonstrated haemoglobin 146g/L, WCC $18.1x10^9$/L with neutrophilia ($16.62x10^9$/L) and a platelet count of $239x10^9$/L. She developed non-immune haemolysis (Direct Coombs test negative, haptoglobin <0.01g/L, total bilirubin 122umol/L and LDH 1136U/L) and thrombocytopenia within two days of admission. Haemoglobin nadir was 67g/L and platelet count $22x10^9$/L. Blood film revealed moderate numbers of schistocytes and spherocytes. There was stage 1 acute kidney injury by KDIGO criteria (creatinine 108umol/L, eGFR 56mL/min). Thrombotic microangiopathy was suspected with no other causative systemic disorders identified. ADAMTS-13 level was mildly reduced to 40% excluding thrombotic thrombocytopenic purpura (TTP). Shiga toxin was not tested in the absence of diarrhoea. Therapeutic plasma exchange (TPE) of 1 Total Plasma Volume (TPV) was initiated on day 3. The patient received 6 sessions of daily TPE with rapid resolution of thrombocytopenia. Laparoscopic cholecystectomy was performed 1 week later. After 3 months of follow up she remains free from relapse.

MAHA and thrombocytopenia secondary to acute pancreatitis is described in approximately 40 case reports. Despite the heterogenous clinical presentations a high index of suspicion, early recognition and timely TPE are emphasised in each series. This case report hopes to raise awareness for this rare, potentially life-threatening complication of a relatively common pathology.

Localised light Chain amyloidosis presentation, treatment and outcomes; an observational study.

Ranjit Anderson N[1,2], Abro E[1], Korcykz D[1,2], Mollee P[1,2]
[1]Princess Alexandra Hospital, Woolloongabba, Australia, [2]University of Queensland, St Lucia, Australia

Aim: To describe the clinical characteristics, treatment modalities and outcomes in patients with localised AL amyloidosis seen at a quaternary amyloidosis referral centre.

Method: Database and medical records for all patients referred between January 2010 to May 2023 with a histologically confirmed diagnosis of localised AL amyloidosis were reviewed. Demographic data, sites of organ involvement, treatment received and recurrence or progression were assessed for each patient. Overall survival was estimated by the Kaplan-Meier method.

Results: 121 patients with localised AL amyloidosis were reviewed with the mean age of diagnosis being 58.4 years; 48% were female. The most common sites of presentation were pulmonary (20%), genitourinary and gastrointestinal tract with 18% each, followed by cutaneous/soft tissue 17%. Other commonly involved sites include the larynx and eye 10% each. Rarer sites were nodal 3%, central nervous system 2%, and nasopharynx 2%.

67% were treated with observation alone following their original biopsy. 21% had surgical management, 4% underwent radiotherapy, 3% received systemic therapy (for localised nodal amyloid associated with lymphoma) and the remaining 7% received other treatment options or had incomplete records. Localised disease recurrence occurred in 14% of cases. There was no progression to systemic AL amyloidosis in any patient. 10% of patients died during the review period, all from causes other than AL amyloidosis. The estimated 5-year overall survival was 91% (95% CI 83–96%) compared to 64% (95% CI 54-73%) for systemic AL amyloidosis.

Conclusion: Localised AL amyloidosis is a distinctively different entity which needs to be distinguished from systemic AL amyloidosis. It has comparatively different presenting organ involvement patterns and a favourable natural history profile without active intervention in the majority of cases. Localised site recurrences occur and can be managed through minimally invasive surgical approaches if symptomatic.

Global seroprevalence of neutralizing antibodies against adeno-associated virus (AAV) serotypes of relevance to gene therapy

Rasko J[1], Bashirians G[2], Petropoulos C[3], Wrin T[3], Paliwal Y[4], Henstock P[5], Somanathan S[2], Casey M[4], da Fonseca Pereira C[6], Winburn I[7], Chabra A[8]
[1]Gene and Stem Cell Therapy Program Centenary Institute, The University of Sydney, Camperdown, NSW, Australia; Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia; Cell & Molecular Therapies, Royal Prince Alfred Hospital, Sydney, NSW, Australia, Camperdown, Australia, [2]Pfizer Inc., Cambridge, USA, [3]Labcorp, South San Francisco, USA, [4]Pfizer Inc., Collegeville, USA, [5]Pfizer Inc., Andover, USA, [6]Pfizer Australia, Sydney, Australia, [7]Pfizer Ltd., Walton on the Hill, UK, [8]Pfizer Inc., New York, USA

Aim: Pre-existing neutralizing antibodies (NAbs) against AAV serotypes can interfere with target cell transduction by rAAV vectors. We performed a multi-country, observational, retrospective, cross-sectional study to assess the prevalence and titre levels of 9 serotypes of NAbs in adults (≥16 years) and children (<16 years). This summary includes results for 6 serotypes (AAV1, AAV5, AAV6, AAV8, AAV9, AAV-Spark100).

Method: Participants from 10 countries (Australia, Canada, France, Germany, Italy, Japan, South Korea, Spain, UK, US) were enrolled from clinical studies conducted 2015–2019, with serum obtained from the Pfizer Biobank. Having optimized the assay, serum samples were diluted and analysed by a central laboratory to determine the NAb titre.

Results: A total of 552 samples were analysed (59% male; <16 years, n=50; 16–40 years, n=95; 41–60 years, n=407). The primary analysis of the overall prevalence of NAb positivity in adults showed that the most prevalent NAb at a dilution of 1:1 was AAV1 (74.9%), AAV6 (70.1%) and AAV5 (63.9%). AAV5 had the lowest seroprevalence. Similar to adults, the primary analysis in children revealed that NAbs against AAV1 were the most prevalent and AAV5 the least prevalent. Overall, samples in children exhibited lower seroprevalence compared with adults. Co-prevalence of NAb positivity was most frequently observed for AAV1 and AAV6, whereas co-prevalence of AAV5 with the other AAVs was less common. South Korea had the highest NAb prevalence; Japan, Australia, and the US had the lowest. The prevalence of NAbs in adults was observed to be higher in females, Asians, and older individuals. Exploratory findings suggest variation in the prevalence of NAbs by biological sex, ethnicity, age, and clinical disease area.

Conclusion: This large, global study of adult and paediatric participants from 10 countries provides new insights into the prevalence of NAbs against a range of clinically relevant AAV serotypes.

A case of erythropoietic protoporphyria treated with an allogeneic stem cell transplantation

Rigano J[1]
[1]*Northern Health, Epping, Australia*

Background: Porphyrias are a group of metabolic disorders caused by enzyme defects in the haem biosynthetic pathway. Biochemical hallmark of porphyrias is overproduction and overexcretion of porphyrin precursor compounds. Erythropoietic protoporphyria (EPP) is caused by deficiency in mitochondrial enzyme ferrochelatase (FECH). FECH inserts ferrous iron into protoporphyrin forming haem. Major site of protoporphyrin overproduction is BM. Protoporphyrin is removed by hepatic excretion and accumulation has propensity for pigmentary liver cirrhosis. Liver transplantation (OLTX) alleviates symptoms of chronic liver cirrhosis while stem cell transplantation (SCTX) corrects underlying defect. This case describes a woman diagnosed with EPP who developed liver cirrhosis and treated with allogeneic SCTX.

Methods: May 2011, a 21-year-old female with EPP and liver cirrhosis presented with chest and abdominal pain, fever with haemoptysis, thrombocytopenia and worsening liver enzymes. She was diagnosed at 18-months-old, protected from sunlight all her life and asymptomatic until December 2010. She suffered refractory RUQ pain, jaundice, splenomegaly, nausea, vomiting and anxiety. She received RBC transfusions, plasmapheresis and haem arginate to manage symptoms. She urgently required OLTX prior to consideration of SCTX.

Results: November 2011, she received a OLTX then fortnightly exchange transfusions and plasmapheresis for six years. Regular liver biopsies showed EPP recurrence and progression, cholestasis, cholangitis, biliary injury, cirrhosis and stage 4 haemosiderosis. March 2017, she received a second OLTX and splenectomy. May 2017, BMAT revealed dyserythropoiesis and 18% ring sideroblasts. August 2017, she received a SCTX however poor chimerism resulted in graft loss. February 2018, she received a second SCTX from the same donor. February 2019 she was in complete haematological remission receiving no exchange transfusion or plasmapheresis in last 12 months. Protoporphyrin, bilirubin and liver enzymes were normal and she has had no restrictions to sun exposure.

Conclusions: Here reported was a woman with EPP successfully treated with a OLTX and SCTX.

Inherited protection against malaria; A case of co-existing sickle cell disease, alpha thalassaemia, G6PD deficiency and Duffy null phenotype.

Rigano J[1], Lee N[1]
[1]*Northern Health, Epping, Australia*

Introduction: The high mortality and widespread impact of malaria have resulted in genetic resistance to this disease and the strongest evolutionary selective force in recent human history. There is extensive overlap of the historical geographical distribution of malaria and human genetic variants that confer resistance to malaria such as sickle cell anaemia, thalassemia, G6PD deficiency and ovalocytosis. This report describes four co-inherited mutations in a pregnant woman that confers protection against malaria.

Methods: A 29-year-old women who was 18 weeks pregnant presented for antenatal testing. Routine haematology, biochemistry, viral serology and haemoglobin electrophoresis analysis was performed. Follow-up testing was performed at 34 weeks and the patient exhibited signs of pre-eclampsia. Magnesium sulphate was administered and RBC transfusion. The woman delivered at 39 weeks without complications.

Results: FBE and blood film revealed a microcytic hypochromic anaemia with NRBCs, occasional sickle cells and schistocytes and macrothrombocytopenia. Haemoglobin electrophoresis detected Hb S (69.9%), Hb F (28.1%) and HbA_2 (2.0%). Molecular analysis confirmed sickle cell disease and alpha thalassaemia. Ferritin and LD were elevated and proteinuria and G6PD deficiency was detected. RBC phenotyping detected Duffy Null confirmed by genotyping. The genetic mutations detected in this woman have produced protection mechanisms against malaria. Hb S impairs *P. falciparum* RBC invasion and growth under low oxygen tension conditions and reduces pathogenicity of *P. falciparum* due to reduced expression of PfEMP1. Alpha thalassaemia reduces pathogenicity through resetting and immunologically primes through cross-species immunity. G6PD deficient RBCs are more fragile to malaria-induced oxidative damage reducing parasite growth rate and causing a more efficient phagocytosis of infected RBCs. The Duffy antigen on RBCs is used by *P. vivax* and *P. knowlesi* as a receptor to mediate its entry into the RBC and its absence prevents parasite entry.

Conclusions: Here reported was a pregnant woman who co-inherited four different mutations that confers protection against malaria.

COVID-19 hyperimmune globulin (Aegros) for the prevention of hospitalisation in patients with immunocompromise: a protocol

Rozen L[1], Smallcombe R[1], Mahler S[1,2], Vijayakumar S[1], Nair H[1,2]
[1]Aegros, Macquarie Park, Australia, [2]Australian Institute for Bioengineering and Nanotechnology, University of Queensland, St Lucia, Australia

COVID-19 continues to cause morbidity and mortality, especially for those with immunocompromise12. Increased immune evasion from SARS-CoV-2 variants of concern (VoC) has diminished the effectiveness of monoclonal antibodies and vaccines; and antivirals can interact with drugs used in these patients . Immunocompromise can blunt vaccine response despite repeated vaccination. Hyperimmune immunoglobulin (HIg) has a long history of use as passive immunity against viruses. Early administration of high titre COVID-19 convalescent plasma (CCP) appears to reduce risk of disease progression in patients with COVID-193. A clinical trial to evaluate efficacy and safety of Aegros' COVID-19 HIg is planned in patients with immunocompromise.
The study is a randomized, placebo-controlled study to evaluate the safety and effectiveness of SARS-CoV-2 hyperimmune globulin in immunocompromised outpatients with less than seven days of symptoms and proven COVID-19. The study will compare standard of care for these patients to standard of care plus a standardized dose of COVID-19 HIG.
The primary end point will be cumulative incidence of hospitalisation or COVID-19 related deaths prior to hospitalisation within 28 days. Safety end points will include cumulative incidence of SAEs and TEAEs. Secondary endpoints will include the pharmacokinetics of a subgroups of patients, the rate of RNA positivity with time and symptom duration.

The study will provide important information about this population of patients who still have a significant unmet medical need.

[1] Baek MS et al, https://doi.org/10.1371/journal.pone.0257641
2 Bahremand T et al, https://doi.org/10.1016/j.lana.2023.100461
3 Joyner MM et al, DOI: 10.1056/NEJMoa2031893

Long-term Safety and Efficacy of Pegcetacoplan Treatment in Adults With Paroxysmal Nocturnal Haemoglobinuria (PNH)

Szer J[1], Peffault de Latour R[2,3], Bogdanovic A[4], Griffin M[5], Kelly R[5], Maciejewski J[6], Mulherin B[7,8], Patriquin C[9], Roeth A[10], Selvaratnam V[11], Savage J[12], Horneff R[13], Tan L[13], Yeh M[12], Panse J[14,15]

[1]Peter MaCallum Cancer Centre, Melbourne, Australia, [2]French Reference Center for Aplastic Anemia and Paroxysmal Nocturnal Haemoglobinuria, Paris, France, [3]Assistance Publique–Hôpitaux de Paris, Saint-Louis Hospital, Université Paris Cité, Paris, France, [4]Clinic of Hematology, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [5]St. James University Hospital, Leeds, UK, [6]Taussig Cancer Institute, Cleveland Clinic, Cleveland, USA, [7]Hematology Oncology of Indiana, Indianapolis, USA, [8]Ascension St. Vincent Carmel, Carmel, USA, [9]University Health Network, Toronto General Hospital, Toronto, Canada, [10]West German Cancer Center, University Hospital Essen, Essen, Germany, [11]Ampang Hospital, Ampang, Malaysia, [12]Apellis Pharmaceuticals, Waltham, USA, [13]Swedish Orphan Biovitrum AB, Stockholm, Sweden, [14]Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), , Aachen, Germany, [15]University Hospital RWTH Aachen, Aachen, Germany

Aim: This analysis aims to evaluate the long-term efficacy and safety for pegcetacoplan in adults with PNH over an additional 48-weeks follow-up during Study 307, the open label extension study for patients previously enrolled in pegcetacoplan international clinical trials (NCT03531255).

Method: 48-week data is presented for patients who completed Phase 1 (PHAROAH, PADDOCK), Phase 2 (PALOMINO), and Phase 3 (PEGASUS, PRINCE) trials. At baseline of the parent studies, patients had haemoglobin <10.5 g/dL despite ≥3 months of stable eculizumab dosing (PHAROAH, PEGASUS) or were complement inhibitor naive (PADDOCK, PALOMINO, PRINCE). Descriptive statistics were used to summarise results for haemoglobin, lactate dehydrogenase (LDH), FACIT-Fatigue, transfusion avoidance (no transfusions required during the 48 weeks; patients who withdrew did not meet the criteria); haemoglobin and LDH normalisation; and mean change in haemoglobin, LDH and FACIT-Fatigue (post hoc). Safety: incidence of any adverse events (AEs).

Results: 137 patients entered the extension study; 107 received 48 weeks of treatment at data cut-off. From parent study baseline to study 307 entry: mean haemoglobin levels improved (and remained stable through week 48); median LDH decreased in baseline complement inhibitor naive patients; mean (SD) FACIT-Fatigue of 34.1 (11.08) increased to 42.8 (8.79) and remained stable. In the total population: 40.2% had haemoglobin >12 g/dL and 31.8% had sex-specific haemoglobin normalisation; normalisation of LDH occurred in 67.0%, and 83.2% had transfusion avoidance.

Overall, 73.7% of patients had an AE through week 48; 16.1% were judged as pegcetacoplan-related; serious AEs were reported in 19.7% (none considered treatment related); 10.9% experienced injection site reactions (mostly mild and transient). No thrombotic events or meningococcal infections were reported. Three patients discontinued the study due to an AE of haemolysis.

Conclusion: Pegcetacoplan sustained robust improvements in haemoglobin, LDH, and fatigue in patients with PNH, and reduced the need for transfusions. Haematologic parameters can be normalised with pegcetacoplan. Long-term safety data corroborate the safety profile reported previously.

Determining the clinical significance of the Monocyte Distribution Width (MDW) on the Sysmex XN-9000 haematology analyser in a cohort of patients with radiologically proven community-acquired pneumonia.

Verran J[1], Faulhaber G[1]
[1]Gold Coast University Hospital, Gold Coast, Australia, [2]Waikato Hospital, Hamilton, New Zealand

Aim: Community Acquired Pneumonia (CAP) is a common respiratory illness that leads to hundreds of hospital presentations in the Waikato region every year. CAP varies in severity from a mild respiratory illness to a life-threatening illness requiring hospital admission due to septic shock and/or requirement for respiratory support.

The primary objective of this retrospective study is to determine whether the Monocyte Distribution Width (MDW), a cell population data parameter on the Sysmex XN-9000 haematology analyser, can predict severity of CAP and therefore be used as a prognostic marker for patients on initial presentation to the emergency department. Other secondary objectives of the study included whether there is a correlation between the MDW, and CURB-65 score in patients with CAP, and to determine whether the MDW correlates with patient outcomes (including length of hospital stay, admission location, complications, inpatient and 30-day mortality).

Method: 382 patients meeting inclusion criteria who had presented to Waikato Hospital between August 2021 and August 2022 were identified retrospectively via the Waikato Hospital clinical coding team.

Data collected on each patient included the full blood count and MDW results from the Sysmex XN-9000 haematology analyser, both at initial presentation and 48 hours after admission (if available). Patient admission data collected included place of admission, length of hospital stay, complications, CURB-65 score, inpatient mortality and 30-day mortality.

The data is currently being analysed using SPSS Statistical software to determine if the MDW can predict both severity as well as patient outcomes in our cohort of patients with radiologically proven CAP.

Next generation sequencing testing identifies the genetic variants in uncharacterised inherited anaemias

Viswanathan G[1], Dass J[1], Dhawan R[1], Aggarwal M[1], Kumar P[1], Seth T[1], Mahapatra M[1]

[1]All India Institute of Medical Sciences (Aiims), New Delhi, India, New Delhi, India

Aim: Inherited anaemias (IA) have a genetic basis. A proportion of IA remain uncharacterised even after extensive laboratory testing; This study aims to evaluate the utility of next generation sequencing (NGS) methods using targeted gene panels for genetic diagnosis of such cases.

Method: Retrospective analysis of IA patients presenting to the hematology clinic was performed. Initial laboratory tests included: hemogram with RBC indices, peripheral smear, reticulocyte count, LDH levels, bilirubin levels, etc. Further tests included Hb HPLC, incubated OFT, 5' EMA dye binding test, G6PD screening test, ARMS-PCR, GAP-PCR and electron microscopy. Bone marrow examination was performed in select cases. Patients of inherited anaemia who still remained uncharacterised were included for further evaluation. 23 of such cases were further studied by targeted NGS with a panel of genes associated with anaemia. The variants were categorised as per ACMG guidelines.

Results: 12-cases (n=12, 52%) showed the presence of a genetic variant. Anaemia subtypes and the corresponding genetic variants identified are as follows: (A) RBC membranopathies & channelopathies (hereditary spherocytosis, hereditary pyropoikilocytosis and sitosterolaemia :*SPTA, PIEZO1, ANK1, ABCG5*); (B) RBC enzymopathies (hexokinase deficiency, pyruvate kinase deficiency :*HK1, PKLR*); (C) □-thalassemia :*HBB*; (D) CDA-II :*SEC23-B*; and (E) other disorders with anaemia (Primary hypertrophic osteoarthropathy and Autosomal visceral heterotaxy-8).

Conclusion: NGS methods are useful to identify genetic basis of otherwise uncharacterised IA. Using a limited gene panel, this study identified genetic aetiology in over 50% of the patients. Routine tests do not characterise all cases of anaemia. Red cell based tests (Hb HPLC, enzyme assays) cannot be performed immediately post blood transfusion. Understanding the genetic basis by NGS leads to better care in these patients. Parents of affected child can be offered antenatal genetic testing in future pregnancies.

Myocardial uptake consistent with cardiac amyloid...now what? A review of nuclear medicine bone scans in an Australian tertiary centre.

Yan S[1], Kempler E[1], Wegner E[1], Bosco A[1]
[1]*Prince of Wales Hospital, Sydney, Australia*

Aim: To review the characteristics and outcomes of patients in whom nuclear medicine bone scintigraphy had detected features consistent with cardiac amyloidosis.

Method: We performed a retrospective audit of patients from a database of nuclear medicine bone scintigraphy at a single Australian tertiary centre between 2008 and 2021. The terms “amyloid” and “amyloidosis” were used in the initial search. Patient characteristics and their outcomes were collated and analysed

Results: The search yielded 40 patients who had features in keeping with cardiac amyloid on nuclear medicine bone scans. The most frequent indication for the scan was clinical suspicion of cardiac amyloidosis. Interestingly, 27 (67.5%) had abnormal findings on transthoracic echocardiography. Few patients underwent cardiac magnetic resonance imaging (7.5%), all of whom had enhancement consistent with cardiac amyloid. Of the four tissue biopsies performed, three were suggestive of amyloidosis. Genetic sequencing was only performed in two patients, neither of which were diagnostic.

Conclusion: Nuclear medicine bone scintigraphy provides an accessible and non-invasive approach to support the diagnosis of cardiac amyloid. However, our findings reveal the lack of consistency among local clinicians regarding subsequent investigations to substantiate the diagnosis. Here we present an opportunity for a streamlined diagnostic pathway within our local health district to aid identification of patients with this rare and under-recognised condition

FLAER as a Standalone Reagent for Testing of Paroxysmal Nocturnal Hemoglobinuria: A Sensitive and Cost-Effective Approach

Praveen s

Aim:

Peculiar problems are encountered while interpreting flow-based PNH testing using monoclonal antibodies against GPI-linked proteins. We hypothesize that FLAER as a standalone reagent may be equally effective for detecting PNH clones. The present study intends to compare the results of a FLAER alone-based strategy to the recommended FLAER+GPI-linked protein-based approach for applicability in clinical settings.

Method:

EDTA-anticoagulated blood samples from patients were tested for PNH by multiparametric flow cytometry. A conventional panel comprising gating markers (CD45 for WBC, CD15 for granulocytes, and CD64 for monocytes) and a combination of FLAER and GPI-linked markers such as CD24 and CD14, henceforth referred to as the '*Routine Panel*.' Second, a '*FLAER alone Panel*' comprising the gating markers and FLAER was set up. Post-processing, the samples were acquired on BC Navios Ex flow cytometer and analyzed on Kaluza Software v2.1. The presence of a PNH clone was reported at a value of ≥0.01%.

Results:

A total of 209 patients were tested. Both panels found a PNH clone in 20.1% of patients (n=42/209) with a 100% concordance rate. The PNH clone range for granulocytes was 0.01-89.68%, and for monocyte was 0.04-96.09% in the *routine panel*. The range in the *FLAER alone panel* for granulocytes was 0.01-89.61%, and for monocytes, it was 0.01-96.05%. Pearson correlation statistics revealed a significant correlation between the size of the PNH clone of granulocytes and monocytes among the two panels tested (granulocytes r=0.9999, p<0.0001, 95% CI=0.9999 to 1.000; monocytes r=0.9974, p<0.0001, 95% CI=0.9966-0.9980).

Conclusion:

Based on our results, FLAER as a standalone marker is specific and sensitive for identifying PNH clones in granulocytes and monocytes, even for high-sensitivity PNH assay. The proposed '*FLAER alone panel*' panel is efficient and cost-effective for highly sensitive PNH testing in two different cell lineages, especially in resource-limited clinical settings.

Enteral fibre supplementation and allogeneic stem cell transplantation outcomes

Andersen S[1,2], Kennedy G[1], Banks M[1,2], Henden A[1,3]
[1]Royal Brisbane And Women's Hospital, Herston , Australia, [2]University of Queensland , St Lucia , Australia, [3]QIMR Berghofer Medical Research Institute, Herston, Australia

Aim: Reduced gastrointestinal microbial diversity and short chain fatty acid (SCFA) levels during allogeneic stem cell transplantation (SCT) has been linked to higher rates of graft versus host disease (GVHD) and infections post SCT (1, 2). Although enteral nutrition (EN) is routinely required post SCT, use of a fibre containing formula has not been studied. This study evaluated feasibility of prebiotic fibre EN provision and assessed clinical and microbiome outcomes in comparison to standard EN.

Method: This pilot interventional study was conducted at a tertiary Queensland Hospital and recruited 30 adult patients (10 to the standard fibre free EN group, 20 to the prebiotic EN group). The prebiotic EN formula contained inulin, oligofructose, arabic gum, soy polysaccharides, cellulose and resistant starch. Enteral nutrition commenced at 30ml/hr continuously the day after transplantation (11g fibre per day), with the rate increasing to meet nutrition requirements if oral intake declined. Stool samples for microbiome analysis and SCFA levels were collected pre and post feeding. Microbiome analysis was completed with shotgun metagenomic sequencing. Analysis of clinical outcomes was completed with Fishers exact tests.

Results: There was no difference in EN tolerance between groups or in clinical outcomes including GVHD (p=0.709), infections (p=1.0), grade of diarrhoea (p=0.101) and mucositis (p=1.0). Microbial diversity declined in both groups with no difference post EN provision (p=0.73). Post feeding, greater abundance of *Lactobacillus_C* (p=0.017) and *lactobacillus_C paracasei* (p=0.041) was observed in the prebiotic group. The abundance of *lactobacillus_C rhamnosus* and *lactobacillus_C paracasei* increased only in the prebiotic group from baseline (p=0.022 and p=0.026).

Conclusion: Provision of fibre containing EN was feasible post allogeneic SCT and may be associated with greater abundance of SCFA producing bacteria. Further comprehensive approaches to prebiotic fibre supplementation should be trialled in future research to evaluate tolerance and optimise microbiome outcomes.

References

1. Haak BW, Littmann ER, Chaubard JL, Pickard AJ, Fontana E, Adhi F, et al. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. Blood. 2018;131(26):2978-86.
2. Payen M, Nicolis I, Robin M, Michonneau D, Delannoye J, Mayeur C, et al. Functional and phylogenetic alterations in gut microbiome are linked to graft-versus-host disease severity. Blood advances. 2020;4(9):1824-32.

Zoledronic acid induced orbital inflammatory syndrome, an underappreciated complication: a case report

Dickey M[1]
[1]*St Andrews Toowoomba, Rangeville, Australia*

Background: Osteoclast inhibitors in myeloma provide protection from skeletal related complications (1). Osteonecrosis of the jaw and atypical femur fractures are well known although rare events(2). I present a case of Zoledronic acid induced orbital inflammatory syndrome a less recognised serious toxicity.

Case report: A 67-year-old man with IgG-kappa myeloma commenced on induction VRD. The patient underwent the first cycle without ocular toxicity while awaiting dental clearance.

Four days following Zoledronic acid initiation there was progressive right eye pain with transient complete right eye visual loss. Latanoprost was commenced for presumed glaucoma without benefit. Symptoms progressed with right eye proptosis, chemosis and right optic disc swelling.

Right orbital inflammation, optic nerve sheath inflammation and perineuritis was found on neuroimaging, this was not present on staging MRI four weeks prior. CSF revealed Varicella zoster IgG, without circulating CSF DNA and an elevated total CSF IgG with a monoclonal band. There was no radiological evidence of CNS myeloma. Peripheral blood testing for an infective or autoimmune aetiology was unrevealing.

Empirical Ceftriaxone 2g, acyclovir 10mg/kg and methylprednisolone 1000mg was initiated. There was prompt resolution of orbital symptoms within 3 hours of corticosteroid administration. CSF Varicella Zoster serology was available on day 2 and corticosteroids were withheld, orbital symptoms reoccurred 3 days post corticosteroids. Following the negative CSF Varicella DNA level, oral prednisone 50mg was started with prompt symptom resolution. The exclusionary diagnosis of orbital inflammatory syndrome secondary to Zoledronic acid was made. Prednisone was weaned over 6 weeks without recurrence of orbital inflammation. There has been no rechallenge of bisphosphonate therapy.

Discussion: Orbital inflammatory syndrome (OIS) is a rare toxicity of Zoledronic acid(3). It is characterised by inflammation of extraocular structures and is a diagnosis of exclusion. Bisphosphonates trigger inflammatory cytokine release which is hypothesized to cause extraocular inflammation(3). Radiology aids in the diagnosis, orbital biopsy is occasional required. OIS often responds to corticosteroids which are tapered over 6-12 weeks (4).

References

1. Berenson JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. Myeloma Aredia Study Group. N Engl J Med. 1996 Feb 22;334(8):488–93.

2. Black DM, Reid IR, Boonen S, Bucci-Rechtweg C, Cauley JA, Cosman F, et al. The effect of 3 versus 6 years of Zoledronic acid treatment of osteoporosis: A randomized extension to the HORIZON-Pivotal Fracture Trial (PFT). J Bone Miner Res. 2012 Feb;27(2):243–54.

3. Sharma NS, Ooi JL, Masselos K, Hooper MJ, Francis IC. Zoledronic Acid Infusion and Orbital Inflammatory Disease. N Engl J Med. 2008 Sep 25;359(13):1410–1.

4. Harris GJ. Idiopathic Orbital Inflammation: A Pathogenetic Construct and Treatment Strategy: The 2005 ASOPRS Foundation Lecture. Ophthalmic Plastic & Reconstructive Surgery. 2006 Mar;22(2):79–86.

Cognition following Chimeric Antigen Receptor T-cell therapy: A systematic review

Kazzi C[1,2], Kuznetsova V[3,4,5], Siriratnam P[1,2], Griffith S[1,2], Wong S[6], Tam C[4,6], Alpitsis R[1,2], Spencer A[6,7], O'Brien T[1,2], Malpas C[1,2,3,8,9], Monif M[1,2,8]

[1]Department of Neurosciences, Faculty of Medicine, Nursing and Health Sciences, Central Clinical School, Monash University, Melbourne, Australia, [2]Department of Neurology, Alfred Health, Melbourne, Australia, [3]Neuroimmunology Centre, Department of Neurology, Royal Melbourne Hospital, Melbourne, Australia, [4]Department of Clinical Haematology, Peter MacCallum Cancer Centre, Melbourne, Australia, [5]CORe, Department of Medicine, University of Melbourne, Melbourne, Australia, [6]Department of Haematology, Alfred Hospital, Melbourne, Australia, [7]Australian Centre for Blood Diseases, Monash University, Melbourne, Australia, [8]Department of Neurology, The Royal Melbourne Hospital, Parkville, Australia, [9]Melbourne School of Psychological Sciences, University of Melbourne, , Australia

Aim: This systematic review aimed to characterise the cognitive outcomes of patients who received chimeric antigen receptor T-cell therapy.

Method: A systematic search of the literature was performed PubMed, PsycINFO, SCOPUS, EMBASE, Medline, and CINAHL (February 2023). Risk of bias was assessed using the JBI Checklist for Case Reports and the Risk of Bias Assessment Tool for Non-randomised Studies.

Results: Twenty-two studies met inclusion criteria with a total of 1104 participants. There was considerable methodological heterogeneity with differing study designs (e.g., cohort studies, clinical trials, case studies, qualitative interview, and a focus group), measures of cognition (e.g., self-report, neuropsychological measures, clinician assessed/neurological examinations), and longest follow-up time points (i.e., five days to five years).

Conclusion: Results of the studies were heterogenous with studies demonstrating stable, improved, or reduced cognition across differing time points. This suggests that cognitive deficits are common post-infusion and resolve within a few months, however, there is a subset of patients who continue to experience persistent deficits more than a year post-infusion. Future studies are needed to comprehensively analyse cognition using a combination of self-report and psychometric measures following chimeric antigen receptor T-cell therapy both in the acute and chronic settings.

Cognitive and psychological presentation prior to CAR-T therapy: a real-world approach.

Kuznetsova V[1,2,3], Rosenfeld H[1,2], Sales C[2], van der Linde S[1], Roos I[2,3], Roberts S[2,3], D'Aprano F[4], Loi S[5,6], Dowling M[1,7], Dickinson M[1,7], Kalincik T[2,3], Harrison S[1,7], Malpas C[2,3,4], Anderson M[1,7,8]

[1]Centre of Excellence for Cellular Immunotherapy and Clinical Haematology, Peter MacCallum Cancer Centre and the Royal Melbourne Hospital, Melbourne, Australia, [2]Neuroimmunology Centre, Department of Neurology, The Royal Melbourne Hospital, Parkville, Australia, [3]Clinical Outcomes Research (CORe) Unit, Department of Medicine (RMH), The University of Melbourne, Parkville, Australia, [4]Melbourne School of Psychological Sciences, The University of Melbourne, Parkville, Australia, [5]Neuropsychiatry, The Royal Melbourne Hospital, Parkville, Australia, [6]Department of Psychiatry, The University of Melbourne, Parkville, Australia, [7]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Australia, [8]Division of Blood Cells and Blood Cancer, The Walter and Eliza Hall Institute, Parkville, Australia

Aim: The current study aimed to characterise cognitive and psychological status in haematology patients planned for chimeric antigen receptor T-cell (CAR-T) therapy and to examine utility of two screening approaches for detecting cognitive impairment.

Method: Sixty patients underwent a specialist cognitive assessment prior to receiving CAR-T at Peter MacCallum Cancer Centre. Data were obtained from objective psychometric measures, other clinical examinations, patient reported subjective cognitive complaint, and a self-report questionnaire of psychopathology and subjective cognitive function. A subset of patients completed a screening measure of cognition. The receiver operating characteristic (ROC) curve analysis examined utility of cognitive screening approaches. Clinicodemographic characteristics were compared between cognitively impaired and cognitively intact patients using Bayesian methods. Bayes factor (BF_{10}) >3 indicated a statistically significant effect.

Results: According to the clinician's impression or to a purely psychometric approach, 15-16 (25.0%-27.0%) patients presented with evidence of cognitive impairment, with six unique patterns of dysfunction. Prevalence and nature of impairment were comparable to cognitive impairment described in the cancer population. Of those patients who completed a self-report measure of psychopathology, nine (15.8%) were elevated on at least one domain, with no consistent pattern of symptoms identified. Compared with cognitively normal individuals, cognitively impaired patients were more likely to have B-cell acute lymphoblastic leukaemia (BF_{10}=9.30), be younger (BF_{10}=7.76), have bone marrow involvement (BF_{10}=5.18), report history of anxiety (BF_{10}=4.85), or have evidence of psychopathology (BF_{10}=31.30). Screening approaches were not useful in detecting impairment.

Conclusion: The study demonstrated a broad spectrum of cognitive dysfunction and psychopathology in haematology patients referred for CAR-T therapy prior to the infusion. The findings did not support utility of a screening approach for identifying patients with suspected impairment to be prioritized for further cognitive examination. Baseline specialist cognitive evaluation is important for detection and management of cognitive neurotoxicity symptoms that might arise after CAR-T.

Epidemiology and outcomes in patients with sepsis and haematological malignancy: 22 years of binational data

MacPhail A[1], Dendle C[2], Slavin M[3], Weinkove R[4], Bailey M[5], Nalpantidis A[6], Pilcher D[5], McQuilten Z[7]

[1]School of Public Health and Preventative Medicine, Monash University, Melbourne, Australia, [2]Monash Infectious Diseases, Monash Health, Melbourne, Australia, [3]Peter MacCallum Cancer and National Centre for Infections in Cancer, University of Melbourne, Melbourne, Australia, [4]University of Otago Wellington, Wellington, New Zealand, [5]Australian and New Zealand Intensive Care Research Centre (ANZIC RC), Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia, [6]Department of Haematology, Monash Health, Melbourne, Australia, [7]Australia Transfusion Research Unit, School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

Background: Sepsis is a major cause of morbidity and mortality in patients with haematological malignancy (HM), but sepsis epidemiology and outcomes are incompletely defined.

Aim: To describe trends in the epidemiology and outcomes of severe sepsis in patients with HM in Australia and New Zealand (ANZ) from 2000-2022.

Methods: Retrospective cohort study of all adults admitted to ICU in ANZ from January 2000 - December 2022 reported in the ANZ Intensive Care Society Adult Patient Database (ANZICS-APD).

Sepsis was defined according to Third International SEPSIS-3 criteria and/or primary admission diagnosis. Leukopenia was defined as leukocyte count $<1.0 \times 10^9$ cells/uL. Admissions were stratified into 5-year periods from 2000-2019 and a 3 year period from 2020-2022. Descriptive analysis was performed.

Results: Among 295,801 ICU admissions for sepsis, 17,970 (5.7%) occurred in patients with HM. Of these, 10,593 (58.9%) had leukaemia or myeloma and 7,154 (39.8%) had lymphoma. Sepsis accounted for 37% of all ICU admissions for patients with HM compared to 11% of all ICU admissions ($p <0.0001$).

Septic patients with HM had higher SOFA scores (mean 5.3 vs 6.7), and higher in-hospital mortality (30.2% vs 15.9%) compared to patients without HM ($p < 0.001$).

Among septic patients with HM across the study period, there was an increase in age (median 62.2 vs 68.7 years, $p <0.001$) and a reduction in in-hospital mortality 47% vs 24.7% ($p <0.001$). The reduction in mortality associated with year of ICU admission remained significant after adjusting for illness severity measured by SOFA score (OR 0.96, 95% CI 0.959-0.963, $p <0.0001$).

Leukopenia was present in 29.6 % patients with HM and sepsis compared to 1.5% of those without. Mortality was no different between leukopenic and non-leukopenic patients with HM (30.9% vs 30.2%, $p= 0.47$).

Conclusion: Outcomes are improving for patients HM and severe sepsis in Australia and New Zealand.

Viridans group Streptococci bloodstream infection in cancer patients receiving chemotherapy: microbiology, treatment and outcomes over a 10-year period from the Royal Brisbane and Women's Hospital

Sheehy J[1], Scott A[1], Henden A[1,4], Kennedy G[1], Redmond A[2,3], Stewart A[2,3,4]

[1]Department of Haematology and Bone Marrow Transplant, Royal Brisbane and Women's Hospital, Herston, Australia, [2]Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Royal Brisbane and Women's Hospital Campus, Herston, Australia, [3]Department of Infectious Diseases, Royal Brisbane and Women's Hospital, Herston, Australia, [4]Pathology Queensland, Herston, Australia

Aim: We aimed to determine (1) the incidence of infective complications (e.g. endocarditis), (2) the duration and type of antimicrobial therapy received for monomicrobial infection, and (3) antimicrobial resistance patterns of episodes of viridans group Streptococci (VGS) bloodstream infection (BSI) occurring in patients receiving chemotherapy.

Method: All VGS positive blood culture specimens between 2013 and 2022 were retrospectively identified from our pathology database (excluding *S. pneumoniae)*. Only those who had received chemotherapy for haematological or solid tumour malignancies were included. Medical records for VGS-positive cancer patients were accessed for demographic, clinical, microbiological and radiological data pertaining to VGS episodes.

Results: Of 581 patient episodes screened, 202 episodes involving 190 cancer patients were identified. VGS episodes occurred following bone marrow transplant (BMT) in 106 (53%), non-BMT haematology chemotherapy in 77 (38%), and solid organ malignancy chemotherapy in 19 (9%). 188 (93%) episodes occurred during neutropenia. The species most commonly isolated were *S. mitis/oralis* (71%), *S. salivarius* (14%) and *S. sanguinus* (6%). 138 (68%) episodes were secondarily screened for endocarditis by a trans-thoracic echocardiogram (TTE), and a subsequent trans-oesophageal echocardiogram (TOE) in 18 (13%); no definitive cases of endocarditis were observed. Two patients had VGS species isolated from blood for two or more consecutive days and no patients had BSI recurrence within 30 days. Of isolates assessed, 156 (81%) and 110 (91%) were susceptible to penicillin and ceftriaxone, respectively. For monomicrobial BSIs, the median total antibiotic duration was 10 days (IQR 7-14), including a median of 4 days (IQR 2-7) post neutropenia resolution. Thirty-day all-cause mortality was 9%, none of which were related to VGS BSI.

Conclusion: VGS BSI following chemotherapy appears rarely complicated by IE or treatment failure; this may reflect early detection and treatment initiation in an at-risk population. Opportunities to limit routine secondary investigations, such as echocardiography, warrant exploration.

Reactions to Serum Eye Drops – the New Zealand Experience and a Review of the Literature

Badami K[1], McKellar M[1]
[1]*New Zealand Blood Service, Christchurch, New Zealand*

Aim: Understand the side effects of serum eye drops (SED).

Methods:

- Review self-reported reactions in NZ patients treated with 25% SED - autologous, allogeneic (group AB males and females), or both between 2003 and 2023.
1. Focussed literature review.

Results:

The NZ experience:

Doses issued: average/patient 787.5 days-worth of SED.

Self-reported reactions: Three patients (0.3%) reported reactions. All were allergic. All occurred with allogeneic SED. We have detailed information on two patients.

Patient 1: Self-resolving pain, redness, grittiness, light sensitivity, deteriorating vision (no systemic features; no problems with autologous SED). Caucasian female, 60y, multiple allergies including almonds, apricots. The *donor* regularly ate Brazil nuts and almonds. Conclusion: ocular allergy; possible cross reactivity between tree nuts.

Patient 2: Three episodes of nausea and vomiting 2h post-*allogeneic* SED in a 38 years old Chinese woman who felt it running down her throat. No systemic features; no known allergies. The donor was an Iranian man. No recurrence after the patient went on to *autologous* SED. Conclusion: likely gastrointestinal allergy to allogeneic SED.

Literature review: Reactions to SED appear few compared to common transfusion reactions. Patients receiving SED are also few. In contrast to the NZ experience, literature reports involve autologous *and* allogeneic SED, and various concentrations. None appears severe. Notably, no eye or systemic infections are reported.

Conclusions: SED are increasingly used to treat ocular surface disease. Types and frequencies of reactions to SED are poorly understood, but appear infrequent. Possible reasons are: (a) serum is less likely (than other blood components) to cause reactions (b) 'Immunological privilege' makes eyes resistant to reactions (c) SED use has been limited (d) reactions occur but SED use is mostly at home, and reactions are mainly mild - therefore under-reported. Monitoring should continue and be made more robust.

Secretor status and susceptibility to IgG ABO antibody-induced haemolysis

Parker A[1], **Badami K[2]**

[1]University Of Otago, Christchurch, New Zealand, [2]New Zealand Blood Service, Christchurch, New Zealand

Aim: Intravenous immunoglobulin (IVIg) used in a variety of disorders, and blood group O plasma – because they contain IgG anti-A, -B, -AB (IgG ABO antibodies) - may cause haemolysis in non-group O (A, B, AB) subjects. Group A/B secretor substance in the plasma of about 80% of non-O group individuals may reduce this risk.
We investigated if in-vitro haemolysis due to IgG ABO antibodies might be reduced by secretor substance.

Methods: One 3g bottle of IVIg (Intragam P) and known anti-A haemolysin-containing group O plasma with fresh, group AB serum as a complement source, were tested for haemolysis against target group AB RBC - with or without the addition of serum containing secretor substance. Appropriate controls were used. Since RBC Lewis phenotyping can indicate secretor status, we used this to identify secretors and non-secretors. Haemolysis was detected using an adapted haemolysin screening method and quantified using cell button width. Since haemolysis due to IgG ABO antibodies can be extravascular, we tested target RBC by direct antiglobulin testing (DAT).

Results: Anti-A haemolysin-containing plasma induced haemolysis in 8/8 and 12/16 tests where *non-secretor* or *secretor* serum, respectively, was added. Thus, the risk of haemolysis with *non-secretor* serum was 1.33 times higher than in tests with *secretor* serum. On the other hand, IVIg did not induce haemolysis in target RBCs – irrespective of whether serum from secretors, non-secretors, or no serum, was added. DAT results with IVIg were positive whether group AB or O RBC were used.

Conclusion: *Secretors* are potentially at lower risk for IVIg- or group O plasma-related haemolysis than *non-secretors*. IVIg results were inconclusive. Negative haemolysin results may have been from low titres or poor complement fixing ability of IgG ABO antibodies in the IVIg used. Positive DAT results with AB and O RBC may have been due to anti-D (all RBC were D-positive).

Blood transfusion consent: What's changed in the last 10 years?

Bastin K[1], Bielby L[1], Akers C[1], Glazebrook B[1], French R[1], Graham A[1], Daly J[2]
[1]*Blood Matters, Melbourne, Australia,* [2]*Australian Red Cross Lifeblood, Brisbane, Australia*

Background: Patients have the right to consent or refuse blood transfusions. To make an informed decision they must be given adequate information in a language or format they can understand. In 2022 Blood Matters undertook an audit of blood transfusion policy and practices, similar to a 2012 audit.

Aims:

- Identify if health service blood transfusion consent policies are available and consistent with best practice guidelines.
- Determine if there has been an improvement in consent policy and practice since 2012.

Method: 140 hospitals from four jurisdictions were invited to participate in the two-part audit.

- Part A – Audit of policy.
- Part B – Retrospective audit of consent to transfusion documentation.

Results: Part A – Consent policy
In 2022, 95 (97%) health services had a policy for blood component consent. Three health services reported no policy; however, a blood consent statement was in other sources. The elements that make up informed consent, such as who and where consent is documented and discussion points, were more frequently reported in policies in 2022 compared to 2012.
Part B – Consent practice
Eighty-seven health services submitted data for 1,891 patients.
Consent was found for 1,823 (96%) patients. Documentation didn't meet all guidelines (854) 47% of the time. Elements of consent with the lowest compliance:

- Consent duration documented and valid for the transfusion episode – (1,493) 82%
- Fully documented dialogue with the patient – (1,130) 62%
- Interpreter needed and provided – (17/69) 25%

In 2012, valid consent was self-reported by auditors at 75%, however valid consent details were not examined.

Conclusion: Blood transfusion consent policies have improved and the practice audit indicates that consent was found for 96% of patients. Transfusion consent can be improved by ensuring patients are fully informed, documenting the dialogue, completing duration and using interpreters when needed.

Granulocyte transfusion as a bridge to allogenic stem cell transplant in severe aplastic anaemia

Bentley L[1], Nissen A[1], Reardon B[1], Pollard L[1], Omar K[1], Langfield J[1], Robertson M[1], Armytage T[1]
[1]NSW Health Pathology, Gosford Hospital, Australia, [2]NSW Health Pathology, Royal North Shore Hospital, Australia, [3]Westmead Hospital, Westmead, Australia

Introduction: Severe aplastic anaemia (SAA) is an immune-mediated disorder defined by pancytopenia and bone marrow hypocellularity. Immunosuppressive therapy and allogeneic stem cell transplantation (SCT) are the pillars of treatment.[1] Affected patients have significant morbidity and mortality risk from infections due to prolonged severe neutropenia.[2] Granulocyte transfusions (GTX) have been used as supportive therapy for patients with neutropenia and severe infection for over 5 decades, however there is extremely limited data on its efficacy. We report a case of GTX in a patient with SAA as a bridge to SCT.

Case Report: A 21-year-old female presented to hospital with widespread petechial rash and was found to have severe pancytopenia. Bone marrow biopsy confirmed marked hypocellularity with normal cytogenetics and no identifiable pathogenic variant by NGS on targeted myeloid and inherited bone marrow failure gene panels. No PNH clone was detected. Oocyte retrieval prior to commencement of IST was complicated by an intraabdominal haematoma which became infected with subsequent polymicrobial bacteraemia including growth of Moaxella Catarrhalis, Pseudomonas aeruginosa, Enterobacter cloacae and Nakaseomyces glabrata. Soon following this, she developed line-associated staphylococcus haemolyticus bacteraemia. Fluid drained from her haematoma grew multi-drug resistant Enterobacter cloaecae, enterococcus faecium and candida glabrata species. Throughout this period the patient remained severely neutropenic (<0.1x10^9/L), and GTX were commenced with the aim to bridge her to haploidentical SCT. She developed donor specific HLA antibodies requiring rituximab and plasma exchange prior to fludarabine, cyclophosphamide and total body irradiation conditioning. The patient received a total of 32 GTX units from the time of initiation until neutrophil engraftment at day 23 post SCT with continuous antibacterial and antifungal therapies.

Discussion: GTX is a non-TGA approved product made from pooled buffy coats of up to 10 compatible donors with a total neutrophil count of >0.3x10^9/L per unit. In a single retrospective case series of 32 patient with SAA who underwent GTX for severe infection, overall survival was reported as 58% and was strongly associated with haematopoietic recovery.[3] 17% of cases developed HLA alloimmunisation after GTX, similar to our case. More research is needed to understand the efficacy of GTX transfusion in SAA.

References:

- Peffault de Latour R, et al; Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. N Engl J Med. 2022 Jan 6;386(1):11-23.
1. Killick SB, Bown N, Cavenagh J, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol 2016; 172:187.
2. Quillen K, Wong E, Scheinberg P, Young NS, Walsh TJ, Wu CO, Leitman SF. Granulocyte transfusions in severe aplastic anemia: an eleven-year experience. Haematologica. 2009 Dec;94(12):1661-8.

Audit of massive transfusion protocol activations in two tertiary hospitals in South Australia

Brazier R[1], Dodd B[2], Ng T[2], Sinha R[1], Tocchetti R[1]
[1]*Blood, Organ And Tissue Programs, SA Health, Adelaide, Australia,* [2]*SA Pathology, Adelaide, Australia*

Aim: To compare and contrast Massive Transfusion Protocol (MTP) activations in two tertiary hospitals in South Australia in respect to blood and blood product usage

Method: Data from MTP activations in 2021 and 2022 at Hospital 1 (H1) and Hospital 2 (H2) was analysed. The data included MTP activations, MTP packs and hospital unit of activation. The MTP pack outcome (used in full, partially used, unused) and the percentage (%) of each blood product returned was analysed.

Results: There were a total of 768 MTP activations across H1 (391) and H2 (377) during the study period, which resulted in the issue of 987 MTP packs. The majority of activations occurred in the Emergency Department (ED) at both H1 (60.9%) and H2 (43.5%). MTP activations resulted in a total of 258 (26.1%) unused, 516 (52.3%) partially used and 213 (21.5%) fully used MTP packs, and the proportions were similar across the two hospitals (Table 1). Intensive Care was the clinical area with the highest proportion of fully used packs at both H1 (50%) and H2 (29.4%), but the location of unused packs differed between the two hospitals with the highest proportion being in ED at H1 (36.5%) and in the Operating Room at H2 (34%). Over 50% of MTP blood products were returned, with Fresh Frozen Plasma being the most common at both hospitals; H1 (61.9%) and H2 (67.4%), followed by Platelets; H1 (57.9%) and H2 (60.73%). Cryoprecipitate was requested in 20% of packs at H1 and 16% at H2.

Conclusion: The number of MTP activations, total packs and total usage were comparable between the two hospitals, but the usage differed slightly at the clinical area level. Partially used MTP packs are common with Fresh Frozen Plasma being the product most likely to be returned to the Blood Bank.

Table 1:

	H1	**H2**
MTP Activations	391	377
MTP Packs	506	481
Fully used	121 (23.9%)	92 (19.1%)
Partially used	242 (47.8%)	274 (57.0%)
Unused	143 (28.3%)	115 (23.9%)

Balancing the supply of blood and blood related products during and after the COVID 19 pandemic

Cameron J[1], Mitchell K[1], Lillington C[1]
[1]***National Blood Authority, Canberra, Australia***

Aim: Maintain a safe, secure and affordable supply of blood and blood related products to patients in Australia while managing interruption to donor capacity and unusual patterns of demand during and after the COVID-19 pandemic.

Method: Through efficient management of collection and supply contracts, the National Blood Authority (NBA) has successfully navigated several challenges in the supply chain resulting from increased demand for red blood cells coupled with reduced donor attendance.

With levels of plasma collections significantly below estimated targets leading to decreased production of domestic immunoglobulin, the NBA was able to source and review detailed data that allowed intensive monitoring of supply, demand, and inventory trends to ensure it was effectively able to respond to changing patterns. The NBA was able to draw on established relationships with stakeholders and suppliers to maintain a close watch on both local and imported blood products supply should pressures arise because of COVID-19. In 2021 the NBA commenced new arrangements with several imported immunoglobulin suppliers to increase the number and volume of immunoglobulin products available. The NBA carefully managed the allocation of products to new patients, and where appropriate and necessary, successfully switched existing patients to equivalent products.

The NBA has also had a focus on supporting the implementation of several measures to increase both whole blood and plasma donations.

Results: These measures have enabled uninterrupted supply of blood products throughout the pandemic with little to no disruption to patient treatment.

Conclusion: Whilst the COVID-19 pandemic has presented challenges with supply never seen before, effective data monitoring and contract management, alongside good working relationships is key to ensuring Australia continues to maintain a world class supply of blood products. Early activation of risk mitigation measures, when unusual supply or demand patterns are observed, allows for the uninterrupted supply of life-saving treatment to patients.

Macrophage-derived secreted factors support the expansion of cord blood-derived cultured red blood cells in an ex vivo system.

Chiaretti S[1,2], Chong F[1], Venida C[1], Griffiths R[1,3]
[1]Australian Red Cross Lifeblood, Kelvin Grove, Australia, [2]School of Health and Behavioural Sciences, University of the Sunshine Coast, Sippy Down, Australia, [3]Australian Institute of Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

Aim: Red cell transfusion is one of the most used therapies, with the potential of treating several diseases and with increasing applications in manufactured cell and gene therapies. However, blood availability relies on donations. An ex vivo culture system that replicates human erythropoiesis using peripheral blood CD34+ cells has been described. Maximally expanded the culture system can produce millilitre quantities of packed functional mature human adult red cells. Macrophages play a fundamental role in erythropoiesis in vivo. Some studies identified macrophages-secreted factors IL-33, ANGPTL7 and SERPINβ2 supported the expansion of cord blood-derived cRBCs. We aim to confirm the effect of the additional factors in cord blood (CB) cRBC production and assess their use in peripheral blood (PB) cRBC production.

Method: CD34+ cells were isolated from CB and PB and cultured for 21 days with a 3-stage differentiation protocol described by Griffiths et al, 2012. Three independent cultures were set up. Human recombinant IL-33, ANGPTL7 and SERPINβ2 were added to both CB- and PB-derived CD34+ every second day of culture. Cells were counted every day and morphology and enucleation rate were assessed at day 12, 14, 18, 21. **Statistical analysis**: growth curves were analysed by 2-ways Analysis of Variance (ANOVA) and enucleation rate was analysed by Student's T TEST.

Results: CB-derived cRBCs proliferation rate was 3 times higher in presence of the three secreted factors tested compared to control cells, while no changes in proliferation were observed for PB-derived cRBCs. Enucleation rate was approximately 65%, higher than the average published enucleation rate for CB-derived cRBCs.

Conclusion: We confirmed that IL-33, ANGPTL7 and SERPINβ2 enhance CB-derived cRBC production and found that PB-derived cRBC production does not respond to these factors. Further investigations to characterise the pathways involved in this response are underway.

Developing Culturally Appropriate Blood Transfusion Resources for Aboriginal and Torres Strait Islander People in Southern NSW.

Coppins E[1]
[1]*Southern NSW Local Health District, Goulburn, Australia*

Aim: To reduce health disparities through effective and appropriate communication with Aboriginal and Torres Strait Islander patients and families that may require a blood transfusion.

Method: A review was requested of available blood transfusion resources to determine if there were culturally appropriate Aboriginal or Torres Strait Islander consumers in Southern NSW. This derived from general concerns regarding pervasive disparities in care that could be concomitant with race/ethnicity, and investigation of which could better serve the organisation in meeting obligations under the NQHCS.

A decolonising theory in conjunction with a narrative approach was the preferred method to conduct research. Research goals were connected to the practices and needs of these consumers. Qualitative data was collected from yarning with consumers.

A draft was compiled in partnership with Aboriginal and Torres Strait islander consumers. Yarning consultation was afforded with the draft before changes being made and the leaflet being finalised.

Results: A comprehensive review against the NQHCS and yarning with Aboriginal and Torres Strait Islander consumers revealed a lack of culturally appropriate content, and a need for additional resources to be developed.

Conclusion: A comprehensive review of the blood transfusion resources available in Southern NSW revealed a lack of culturally appropriate content. An Aboriginal and Torres Strait Islander information leaflet for blood transfusions was developed in partnership with our consumers. The leaflet meets recommendations from the gap analysis against the NQHCS.
The leaflet will allow clinicians to help provide culturally appropriate care and support. It encourages active dialogues in which patients and providers can ask questions, correct misunderstandings, and build trust. By improving our cultural responsiveness, we not only remove barriers to accessing healthcare, but may also reduce inequitable health outcomes for marginalised and vulnerable groups.

Implementation of upfront use of O positive red cells shows no apparent reduction in use of O negative red cells at Royal Melbourne Hospital vs. The Alfred

Akers C[1], Pickles L[1], Bastin K[2], Webb A[1], Lapenta S[2], Kelsey G[2], Davis A[1]
[1]Laboratory Haematology, The Alfred Hospital, Melbourne, Australia, [2]Laboratory Haematology, Royal Melbourne Hospital, Melbourne, Australia

Aim: Whilst demand for most red blood cell (RBC) groups decreases, use of O negative RBCs increases and supply issues occur. In response, some health services (HS) use O positive RBC upfront for emergency transfusion. This study compared O negative RBC use at The Alfred (A) and Royal Melbourne Hospital (RMH) pre and post RMH implementing O positive RBC use in certain patient groups.

Method: Review all O negative RBC transfusions over two time periods. Period 1: Both HSs using O negative RBC for emergency transfusion. Period 2: RMH transitioned to O positive RBC for emergency transfusion

- Identify number of O negative RBC units transfused per patient blood group.
- All patients not group O negative who received O negative RBC are for further assessment.

Results:

Table 1. O negative RBC use

	Alfred		RMH	
	Sept -Nov 22	Jan-Mar 23	Sept-Nov 22	Jan-Mar 23
No patients	175	176	214	197
O neg issued	567	511	591	659
All RBC issued	4119	3951	3740	3913

Table 2. Total number of O negative RBC transfusions per patient blood group

Patient blood group	Alfred		RMH	
	Sept-Nov 22	Jan-Mar 23	Sept-Nov 22	Jan-Mar 23
A neg/A Pos	26/68	19/54	28/97	43/127
AB neg/AB Pos	0/5	0/5	3/14	6/11
B neg/B Pos	10/29	16/71	17/80	32/70
O neg	262	198	141	196
O pos	167	148	211	174
Total	567	511	591	659

Analysis of indication for O negative transfusion such as Massive Transfusion will be performed.

Conclusion: No reduction in use of O negative red cells was seen following the introduction of O positive RBC for patients with unknown blood group at RMH. Exploration of practices at these institutions which may influence the results, such as pre prepared packs in massive transfusion, is required. Alternate approaches to address shortages of O negative RBCs may be necessary.

Onsite storage of red cells in the emergency department of a metropolitan trauma centre and its effect on massive transfusion protocol activations

Dodd B[1], Tocchetti R[2], Sinha R[2], Ellis D[3]
[1]SA Pathology, Adelaide, Australia, [2]Blood Organ and Tissue Programs, SA Health, Adelaide , Australia, [3]Emergency Department Trauma Service, Royal Adelaide Hospital, SA Health, Adelaide, Australia

Aim: Previous analysis of the Royal Adelaide Hospital Emergency Department Trauma Service (EDTS) data showed onsite EDTS emergency red cell units in a Haemorrhage Starter Pack (HSP) reduces the number of massive transfusion protocol (MTP) activations (HAA Conference 2017). To streamline blood product availability, an EDTS Blood Fridge was installed holding O Neg red cell units (RC). This study assesses how holding onsite blood in EDTS has affected MTP activations.

Method: All MTP activations in the EDTS and EDTS Blood Fridge usage for a 2yr period (Jan 2021 – Dec 2022) were compared with a period before on-site availability of blood. The data included whether the MTP packs were used or returned unused, access of the onsite fridge blood and if this resulted in MTP activation. This data was compared with pre-onsite blood data.

Results: During the study period, EDTS activated 201 MTPs and 258 MTP packs were issued. The EDTS Blood Fridge was accessed on 102 occasions and a total of 169 RC were transfused. Of the 201 activations, 16.7% were fully used (previously 16%), 49.2% were partially used (previously 34%) and 34.1% (previously 49%) unused. 52.1% of red cells, 65.8% of FFP and 64.9% of platelets were returned from the MTPs. Of the 258 MTP packs, 84 packs (32.6%) also initially used EDTS blood fridge red cells. Of these 84 MTP packs, 19 (22.6%) were returned unused (vs 49% previously), 44 (52.4%) were returned partially used and 21 (25.0%) were fully used.

Conclusion: A proportion of the MTP activations were preceded by EDTS blood fridge blood use. This onsite blood assists in the blood management of patients by trauma physicians. Onsite storage of red cells in the EDTS also reduces the number of returned unused MTP packs.

Custom tailoring versus one size fits all: what is the optimal strategy to improve Haematopoietic Stem Cell harvesting?

Duarte G[1], Lewandowski S[2], Wood E[1], Stanbury M[1], Mannion S[1], Wee D[1], Wang M[1], Vera A[1], Reid L[1], Hessel J[1], Meads K[1], Rutledge A[1], Ammari N[2]
[1]New Zealand Blood Service, Christchurch, New Zealand, [2]Terumo Blood and Cell Technologies, Lakewood, USA

Aim: The success of the stem cell transplant relies on optimal stem cell harvesting. Data analysis can optimize the apheresis collections, and help decrease harvesting failure. In this study, we evaluated the results of stem cell collections according to two different approaches: standard procedure target for all collections vs. algorithm with a collection efficiency (CE2) target.

Method: 202 collections from 119 patients and donors submitted to peripheral blood stem cell (PBSC) harvesting from March 2021 to February 2023, at Christchurch Hospital, New Zealand, were retrospectively reviewed. From March 2021 to April 2022, the One Size Fits All (OSFA) approach was utilized (processed volume from 2.0 to 3.0 x total blood volumes (TBV)). From May 2022 to February 2023, the volume to be processed was calculated according to the patients $CD34^+$ collection target and peripheral count (Custom Tailoring (CT) strategy).

Results: In the CT the number of collected cells and procedures per patient shows a trend suggesting improved performance. The proportion of transplants requiring 3 consecutive collection days decreased and the success rate according to the target cell dose increased (Table 1).

Conclusion: The CT strategy promoted better CD34+ collections achieving the desired cell target. Further studies with larger samples and comparing performance between a CT approach based on a CE2 target and a customized prediction algorithm should be performed.

Table 1: Summary information of PBSC	**One Size Fits All**	**Custom-Tailored**	**P value**
Patients (n)	72	47	-
Autologous / Allogeneic	72/0	45/2	-
Age (years)	61.0 ± 16.3	60.0 ± 12.1	0.582
Sex			
Male	45 (62.5%)	27 (57.4%)	0.749
Female	28 (38.5)	19 (40.4%)	0.749
Collections	128	74	-
Collection / Patient	1.76	1.57	0.174
TBV Processed* (range)	3.0 ± 0.3 (1.6 -3.2)	3.0 ± 0.5 (1.1- 4.0)	<0.001
Large Volume Leukapheresis	5 (3.9%)	34 (45.9%)	<0.001
Duration (minutes)	269 ± 44	286 ± 48	0.095
CE2 (%)	46.1 ± 11.4	47.6 ± 10.8	0.896
Pre-$CD34^+$ cells/ µL	23.0 ± 48.4	30.0 ± 56.1	0.273
Product $CD34^+$ cells x10^6/Kg	1.8 ± 5.0	2.9 ± 3.4	0.601
Collection Outcome**			
No failure	78% (46%)	87% (63%)	-
Optimal failure	22% (32%)	11% (20%)	-
Minimal failure	0% (22%)	2% (17%)	-

Quantitative measures were presented in the form of median ± standard deviation. *According to Nadler's formula. ** Collections outcome according to internationally accepted minimal cell dose for an autologous transplant (2.0 x 10^6 $CD34^+$ cells/kg) and to local criteria (minimum dose for one autologous transplant for myeloma 3.0 x 10^6 $CD34^+$ cells/kg; lymphoma, solid tumors and allogeneic transplants 5.0 x 10^6 $CD34^+$ cells/kg). Minimal failure = not enough cells for one transplant; Optimal failure: optimal dose for more than one transplant not achieved; No failure = total dose requested up to 3 transplant achieved.

Management of a rare Anti-Vel antibody in pregnancy

Khoo T[1], Flinn H[1], Lee R[1], Ackerman L[1]
[1]*Department of Haematology, Royal Prince Alfred Hospital, Sydney, Australia*

Aim: To describe the clinical, laboratory and patient blood management issues in a case of a pregnant woman with an anti-Vel antibody at risk for post-partum haemorrhage (PPH).

Method: Vel is a clinically significant, high incidence blood group antigen. Allo-immunisation of the rare Vel-negative individual (1:4000) with Vel-positive red cells results in the development of anti-Vel antibodies that are associated with severe haemolytic transfusion reactions. We describe a case of a lady with an Anti-Vel antibody detected at routine antenatal assessment during her fourth pregnancy. Initial testing revealed a positive antibody screen which displayed pan-agglutination in IAGT and enzyme when tested against an eleven cell panel of fully phenotyped red cells. Preliminary findings suggested a high incidence antibody and further testing performed by Lifeblood confirmed an anti-Vel antibody. In Australia, there was only one known compatible donor identified. In addition, as the patient was pregnant, autologous collection was not possible. The patient's parents were in a consanguineous marriage and out of her seven siblings, only one other sibling was Vel-negative. There was also a limited availability of cryopreserved Vel-negative red cells. Due to these limitations in supply of Vel-negative red cells, a multifaceted alternative care plan was developed, including: (i) Patient optimisation for delivery, including replacement of haematinic factors. (ii) Early anaesthetic and obstetrics involvement was sought in planning delivery. (iii) In the event of PPH, a clearly documented prescription of haemostatic products was suggested including upfront cryoprecipitate, platelets, FFP, tranexamic acid, and recombinant Factor VIIa.

Results: The patient was monitored closely in the high risk obstetric clinic in close collaboration with haematology. The pregnancy and subsequent delivery was uncomplicated.

Conclusion: This case demonstrates the importance of a comprehensive transfusion plan for patients with rare blood groups.

Peptide mimetics as artificial Scianna blood group antigens

Flower R[1]
[1]Australian Red Cross Lifeblood, Kelvin Grove, Australia, [2]University of Sydney, St Leonards, Australia

Aim: The Scianna (Sc) Blood Group system, ISBT 13 with 8 antigens and 2 null variants, is located on chromosome 1 p34-365. The antigens are expressed on the Human Erythrocyte Membrane Associated Protein (ERMAP) a 60-68kD immunoglobulin superfamily glycoprotein. SC1 (Arg57) is high frequency in all populations with SC2 (Gly57) found at low frequency, a null variant SC3 has been reported in Pacific Islanders with other variants considered very rare. RBC for detection of antibodies to the SC2 variant are difficult to obtain and the utility of peptide mimetics in an ELISA to screen for antibodies to SC2 was investigated.

Method: Nine residue SC1/SC2 peptides with the variant at position 5, both N-terminal and C-terminal biotinylated forms, were purchased from Mimotopes Pty Ltd, Australia. These peptides were captured on neutravidin-coated plates and binding of a known anti-SC2 antibody investigated. A number of de-identified antisera discarded after completion of antenatal screening were tested for anti-SC2 antibodies by antiglobulin testing and ELISA. No cases of HDFN were recorded for any of the samples used in this study.

Results: An antibody to SC2 reacted with both SC1 and SC2 peptides in both orientations with the difference in reaction strength not sufficient to resolve SC1/SC2 specificity. Screening of de-identified antenatal sera for antibodies by a standard antiglobulin test revealed weak antibodies to SC2 in 9 of 417 sera tested, with 2 of these also ELISA positive but with both SC1 and SC2 peptides.

Conclusion: Peptide-ELISA may be useful to investigate whether anti-Sc antibodies have been detected, when phenotyped RBC are unavailable, and to screen for new antisera for RBC-typing. It may be of interest to investigate donors in populations in Fiji and PNG for null variants and antibodies to SC3, produced in individuals homozygous for a null phenotype, utilising a peptide-ELISA or a cloned SC extracellular construct.

Collaboration and communication: practice change initiative to reduce O RhD negative red blood cell use in Victoria

French R[1], Bielby L[1], Daly J[2]
[1]*Blood Matters, Melbourne, Australia,* [2]*Australian Red Cross Lifeblood, Brisbane, Australia*

Background: Prolonged shortages of O RhD negative (O neg) red cells (RBC) were experienced across Australia in 2022. 16.2% of RBC issued were O neg (Victorian average to September 2022), 8.7% of new Australian donors are O neg.

Many O neg RBC held for emergency use are transfused to prevent expiry.

The National Blood Authority (NBA) working group developed the use of emergency group O RBC National statement which includes the use of O RhD positive (O pos) RBC.

Aims:

- Implement and support practice change to include emergency use O pos RBC for females >50 years & males >18 years in line with the NBA National statement.
- Reduce O neg RBC demand, supporting availability.

Method:

- Extensive consultation and engagement with pathology providers, jurisdictions already with this policy, Australian Red Cross Lifeblood (Lifeblood), Safer Care Victoria (SCV) and expert clinical committees.
- Develop practice change recommendations and resource suite supporting the National statement.
- Obtain endorsement from Lifeblood, SCV and expert clinical committees.
- Pilot resources, ensuring clear messaging and meaningful clinical language.
- Provide information sessions, facilitate discussion prior to National statement release.

Results: Resources developed and launched (2/3/23) include practice recommendations, educational presentations, emergency protocol guidance, flowcharts, RBC swing tags and audit tool. Hard copies distributed and available for download from Blood Matters webpage.

Since launching (2/3/23) 460 webpage pageviews (to 12/4/23).

SCV distributed communication to >12,500 contacts across Victoria. SCV social media campaign - Twitter 987 views, 10 reposts. LinkedIn 95 likes, 12 shares.

Early engagement resulted in several health services implementing use of emergency O pos RBC, ahead of National and Blood Matters launch. Several thousand views through SCV homepage.

Conclusion: Successful practice change requires time for planning, consultation, and stakeholder engagement along with supporting education and resources.

Post implementation follow-up, development of transparent O neg holdings governance, and undertake state-wide audit.

Fridges and Fridays mock Massive Haemorrhage Protocol (MHP) scenario generator

Gould K[1]
[1]*Sullivan Nicolaides Pathology, Bowen Hills, Australia*

Aim: Develop small scale mock MHP scenarios for multi-discipline scientists that can be contextualise to local settings and used for initial training, refresher training, on-going competency, or purely for practice.

Background: Sullivan Nicolaides Pathology (SNP) has over 20 laboratories across Queensland and northern New South Wales (NSW) that provide transfusion support to surrounding private hospitals and clinics. Scientists who staff the regional SNP laboratories are multi-discipline scientists, continually rotating through the core disciplines of the laboratory including blood bank. There was a desire to have regular mock MHP scenarios that were accessible so the scientific team would feel better prepared and more confident when real MHPs occurred.

Method: Internal logs were reviewed for cases that had occurred at the SNP regional laboratories. A copy of *Dicing with Death* Question Adaptation Guidance (UK NEQAS) was consulted to provide architecture for building mock MHP scenarios. An Excel file with additional coding was built containing de-identified cases broken into 8 different patient demographics (age, sex), 8 different clinical presentation or situation (e.g. in theatre for spinal surgery) and 24 different blood bank results. Blood bank results were further divided into 12 basic and 12 complex types.

Results: A low-resource, small-scale tool for SNP regional laboratories to build confidence and preparedness into their scientific teams for real MHPs was created. At time of this abstract submission, potentially 9 216 mock MHP scenarios can be generated, similar to the format of *Dicing with Death* (UK NEQAS). Prompting questions and 'coaching cues' embedded throughout the timeline of the mock MHP scenarios allowed for scientific staff to become familiar with accessing and using internal MHP policies.

Conclusion: Whilst initially perceived as a 'clunky' way to execute mock MHP scenarios, adapting real-life SNP cases provided authenticity to the scenarios, and allowed for internal MHP policies to be practiced.

References: UK NEQAS Haematology & Transfusion*, Dicing with Death* Question Adaptation Guidance, personal email communication.

Internal SNP MHP logs, on-call logs, interesting cases logs

Reducing red cell wastage – the value of centralised management and surveillance in WA

Graham J[1], Le Viellez A[1], Fong E[1], Finlayson J[1], Kavanagh S[1]

[1]*Pathwest, Nedlands, Australia*

Aim: To reduce red cell unit wastage at PathWest, a public pathology provider operating 27 laboratories in Western Australia, through stock rotation and development of centralised inventory management tools for rapid assessment of blood stocks.

Method: PathWest implemented a red cell wastage reduction programme in May 2022. This programme focused on:

- Centralised inventory management through a central co-ordinator, responsible for facilitating stock rotation and liaison with suppliers during times of shortage/disruption.
- Development of product inventory dashboards. The inventory manager created a dashboard to track inventory across sites, identify stock nearing expiry for preferential transfusion and to identify units for transfer to sites with higher transfusion volumes under a hub-and-spoke inventory rotation model.
- Regular data analysis, allowing trends in wastage to be identified and addressed in a timely fashion.
- Logistics support, particularly for remote sites. Close liaison with external transport companies was required to ensure timely and safe delivery/collection of red cells to reduce wastage without compromising patient/laboratory needs.
- Building effective working relationships across the State through engagement with laboratory staff at all sites.

Results: Significant improvements have been achieved since programme inception. These include:

- Saving a total of 875 red cell units (corresponding to ~$310,000 in production costs) in the financial year to date against a similar period in FY21/22.
- A significant fall in the discards as percentage of issues (DAPI) falling from an average of 4.2% to 1.7%.
- An 84% reduction in the number of red cell units being discarded through expiry. Expiry of group O RhD-negative units fell by 91%.

Conclusion: Establishing a centrally co-ordinated inventory management programme has resulted in major reductions in red cell wastage, conserving blood stock in Western Australia and resulting in cost savings for the State. Work to embed these processes, and to address non-laboratory sites with red cell inventory, is ongoing.

Isolation and characterisation of extracellular vesicles from cryoprecipitate

Hwang J[1,2], Tung J[1,2,3], Flower R[1,2], Harkin D[1,2], Pecheniuk N[1,2]
[1]Queensland University of Technology, Kelvin Grove, Australia, [2]Australian Redcross Lifeblood, Kelvin Grove, Australia, [3]University of Queensland, Brisbane, Australia

Aim: Extracellular vesicles (EVs) are nano-sized membrane particles released from most cell types and play an essential role in intercellular communication. They can be isolated from many biological fluids, including blood components. This study aims to characterise the EVs in cryoprecipitate, a clinical vital blood component derived from fresh frozen plasma (FFP) that is largely unknown in the EV field.

Method: Individual cryoprecipitate units were obtained from Australian Red Cross Lifeblood, and EVs were isolated using size-exclusion chromatography. The purified EVs were then characterised using different techniques based on the recommendations set forth by the International Society for Extracellular Vesicles (ISEV) for the minimal information for studies of extracellular vesicles (MISEV2018). Nanoparticle Tracking Analysis was utilised to determine the size distribution and concentration. At the same time, Western blotting was employed to validate the isolated vesicles for the common EV-enriched markers. In addition, we used transmission electron microscopy to perform structural characterisation of the EVs.

Results: EVs in cryoprecipitate had a size ranging from approximately 30-200 nanometres in diameter, with a cup shaped morphology when observed using TEM. In addition, western blot analysis confirmed the presence of common EV markers such as CD9, CD81 and flotillin-1 in these vesicles, which differ from those found in FFP.

Conclusion: To the best of our knowledge, this was the first study to isolate and characterise EVs in cryoprecipitate based on MISEV2018 guidelines.

Blood group genomics and phenotype/genotype reference sources

Hyland C[1,2], Lopez G[1,3], Flower R[1,2], Gassner C[4]
[1]Australian Red Cross Lifeblood, Kelvin Grove, Brisbane, Australia, [2]Faculty of Health, Queensland University of Technology, Kelvin Grove, Brisbane, Australia, [3]School of Health, University of the Sunshine Coast, Sippy Downs, Sunshine Coast, Australia, [4]Private University in the Principality of Liechtenstein, , Liechtenstein

Aim: Blood group genomic studies have contributed to the discovery of blood group systems and antigens, to solving complex blood group problems and to enabling large scale population studies. The latter is expanding our knowledge of blood group profiles across diverse populations. Blood group genomics, however, is dependent on accurate and well-maintained reference sources that link genotype with predicted blood group phenotype.

Herein we review the past and ongoing role for the Blood Group Antigen and Allele Tables provided as a volunteer professional service by immunohematology experts within the Working Party (WP) of the International Society of Blood Transfusion (ISBT).

Methods: Review of ISBT Working Party reports published between 1980 and 2022.

Results: In 1980 the ISBT established a system 'to devise a numerically based, internationally agreed, terminology for red cell antigens'. [1] In 2008 this was expanded to include a 'sub-committee for new terminology for blood group genes and alleles; for use in transfusion field; with alleles listed in ISBT website'. This sub-committee is now called the WP for Red Cell Immunogenetics and Blood Group Terminology.

Up to June 2023, successive members of this WP have registered 44 blood group systems comprising over 350 antigens*. Over 1800 blood group genetic variants associated with defined blood group phenotypes have been curated. Blood group genomic studies within Australia have contributed recently to five antigen discoveries and to multiple alleles. Finally, global population studies are also revealing a further layer of blood group genetic variants of unknown phenotype significance.

Conclusion: The foundation laid by serologists and immunohematologists from the late 20th is now a critical resource for blood group genomics. The ongoing presentation of blood group phenotype and genotype data is expected to evolve further to accommodate the ongoing developments from blood group genomic studies.

1. Allen F. H., Anstee D.J., Bird G.W.G., Contreras M., Crookston M., Engelfriet C.P., et al. ISBT Working Party on Terminology for Red Cell Surface Antigens. Vox Sanginis. 1982;42:164-5.

* Red Cell Immunogenetics and Blood Group Terminology | ISBT Working Party | The International Society of Blood Transfusion (ISBT) (isbtweb.org) Accessed 2nd June 2023

A re-audit of post-partum Anti D use and Kleihauer testing in New Zealand - did we improve?

King F[1]

[1]New Zealand Blood Service, Wellington, New Zealand

Kleihauer testing is recommended [1,2] following a potential sensitising event after 20 weeks' gestation to detect large fetomaternal bleeds which may require additional doses of RhD Immunoglobulin to prevent immune anti-D antibody formation. In a 2009 audit[3] on the use of RhD immunoglobulin in the eight largest District Health Boards (DHBs), less than half of the women (44%) were Kleihauer tested following an antenatal indication after 20 weeks or at birth.

Aim: To ascertain, in RhD negative women following the birth of an RhD positive or unknown baby, 1. the level of Kleihauer testing completed, 2. the level of RhD Immunoglobulin use and dosing comparing this to the results obtained during the 2009 Clinical Audit of RhD Immunoglobulin in New Zealand (NZBS).

Method: Data was collected from nine DHBs across New Zealand, covering 74.9% of births nationwide utilizing information collected from the Ministry of Health and the New Zealand Blood Service.

Results: 10% of the 40,405 women who gave birth between 1 July 2018 – 30 June 2019 were RhD negative. Two thirds of those had a baby that was RhD positive or RhD unknown. Kleihauer testing was performed in 76% of these women , a significant improvement on the 2009 audit.

96% of women received RhD immunoglobulin perinatally. That leaves approximately 87 RhD negative women with RhD positive or unknown babies unprotected from sensitisation at birth. In this audit, this lack of RhD Immunoglobulin administration represents a significantly higher risk for the formation of anti-D antibodies than that due to lack of Kleihauer testing. A small proportion of women did not have blood groups identifiable through the audit's methodology but an expected number did receive RhD Immunoglobulin, suggesting these women were appropriately cared for.

Conclusion: All audited DHBs now have policies in place requiring Kleihauer testing for RhD negative women giving birth to RhD positive or RhD unknown babies. Kleihauer testing remains the principal test used across all DHBs with only two offering HbF flow cytometry

References:

1. Women's Health Committee. Guidelines for the use of Rh(D) Immunoglobulin (Anti-D) in obstetrics. Guideline https://ranzcog.edu.au/statements-guidelines/obstetrics/rhdimmunoglobulin-(anti-d)-in-obstetrics-in-austr (2019).
2. New Zealand College of Midwives. Consensus Statement: Anti-D prophylaxis administration during pregnancy and early postpartum. https://www.midwife.org.nz/wp-content/uploads/2021/07/Anti-D-prophylaxis-duringpregnancy-and-early-postpartum.pdf (2021).
3. King, F., Thrift, L. & Charlewood, R. A Clinical Audit Of RhD Immunoglobulin In New Zealand. https://www.clinicaldata.nzblood.co.nz/resourcefolder/audits/AntiD.Audit.final.report.pdf (2011).

Red Blood Cell Alloimmunization and Autoimmunization in Blood Transfusion-Dependent Sickle Cell Disease and β-Thalassemia Patients

Kuriri F[1]
[1]*Shaqra Univeristy, Shaqra, Saudi Arabia*

Aim: Sickle cell disease and thalassemia are highly prevalent in the Al-Ahsa Region of Saudi Arabia, leading to a large number of patients requiring multiple transfusions. The risk of developing transfusion-related complications, especially alloimmunization, is an ongoing concern for transfusion-dependent patients. It is important to determine the rate of alloimmunization and autoimmunization in Al-Ahsa Region, Saudi Arabia, where sickle cell disease (SCD) and thalassemia incidence rates are the highest in Saudi Arabia

Method: A cross-sectional study was conducted to review the transfusion history of patients with SCD and thalassemia at the King Fahad Hospital (KFH) in Al-Ahsa, Saudi Arabia. 364 transfusion-dependent patients were included in this study.

Results: Alloimmunization rates in patients with SCD and thalassemia were 16.7% and 11.97%, respectively, while autoimmunization rates in patients with SCD and thalassemia were 5.3% and 0.7%, respectively. The most frequent alloantibodies among the study participants were against Kell, Rh blood group systems.

Conclusion: Blood transfusion-related alloimmunization and autoimmunization compromise the proper management of chronically transfused patients. Ideally, extended matched phenotyping should be implemented to prevent alloimmunization and reduce the risk of developing blood transfusion-related alloantibodies.

The Effect of Haematocrit on the Composition and Viscoelastic Properties of Ex-Vivo Thrombus Formation

McGuire C[1], Bulmer A[1], Pritchard R[1], Hicks A[1]
[1]*Griffith University, Tweed Heads, Australia*

Background: Red blood cells have traditionally been viewed as passive participants in blood clotting. However, recent studies indicate that haematocrit appears to influence thrombosis risk, blood clot structure, and their mechanical properties. The latter will affect the success of various interventions to remove them. This study aims to investigate the role of haematocrit in ex vivo thrombus formation and the properties of blood clots as haematocrit deviates from normal.

Method: Whole blood was collected from participants (n=25) and altered to set haematocrits (10-50%), whilst controlling for platelets. Thrombus properties were measured using a ClotPro™ device for each haematocrit dilution, measuring maximal clot firmness (MCF), clot formation time (CFT), clotting time (CT), and maximum lysis (ML). Clots were produced *ex-vivo* in an artificial vessel for histological analysis. Clot composition was characterized by using Haematoxylin & Eosin, and Martius Scarlet Blue stains, as well as anti-CD42b and anti-FGG immunostains.

Results: Clot formation time increased significantly between 10% (85.71s ± 23.95) and 50% haematocrit (223.1s ± 56.17) (p=0.0001). Maximum clot firmness was highest in the 10% haematocrit samples (59.13mm ± 4.523), with 50% haematocrit being the least firm (49.16mm ± 3.793) (p=0.0001). Histology revealed larger proportions of 'white' areas that were more prevalent in low haematocrit clots. Platelet-neutrophil adhesion and areas of increased platelet-fibrin deposition were observed, which may further explain their resistance to clinical interventions.

Conclusion: The findings of this study support the growing understanding that red blood cells play a role in clotting by reducing firmness and increasing clot formation time. Supporting the theory that the efficacy of certain treatments will vary depending on the composition of the clot. Additionally, this study's development of a model of ex-vivo blood clot formation may aid the development of future interventions to test clot extraction and medical devices to reduce the burden of thrombotic disease.

Transfusion Management for Patients taking an Anti-CD38 (Daratumumab) Monoclonal Antibody at Central Blood Transfusion Service Indonesian Red Cross- Jakarta- Indonesia

Merizka E[1]
[1]CBTS IRC , South Jakarta, Indonesia, [2]University of Muhammadiyah Prof. Dr.Hamka, South Jakarta, Indonesia

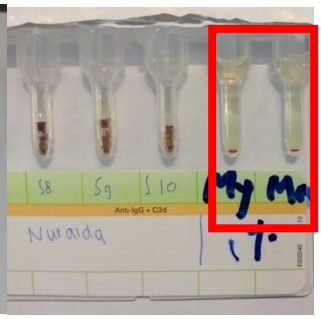
Aim: This study aims to see whether 0.2 M DTT can interrupt the attachment of Daratumubab to CD 38 red blood cells of multiple myeloma patients.

Method: Serum and red blood cells samples from one patients on anti-CD38 (Daratumumab) monoclonal antibody treatment were evaluated. Tests performed included ABO/RhD typing, indirect antiglobulin test, direct antiglobulin test and eluate test. A daily evaluation was performed to determine the shelf life of dithiothreitol-treated red blood cells when stored in Alsever's solution. The red blood cells of multiple myeloma patients and the patient's donor red blood cells were treated using a 0.2 M DTT. A pre-transfusion test was carried out before and after treatment using a 0.2 M DTT. The method used in this case report is the gel test method.

Results: The initial hypothesis of the study was that there were differences in the results of the pre-transfusion test of multiple myeloma patients with their donors before and after being treated with DTT 0.2 M. In the pre-transfusion test results prior to treatment with DTT 0.2M, major and minor incompatible results were obtained on examination and positive results on the DCT and ICT test results. After treatment on the red blood cells of the patient and the donor with a DTT of 0.2 M, compatible results were obtained in the pre-transfusion test.

Before treatment with DTT 0.2M

After treatment with DTT 0.2M



Conclusion: Treatment of red blood cells with dithiothreitol 0.2M can be efficient and accessible to offset the interference of the anti-CD38 (Daratumumab) drug in pre-transfusion tests. The number of costly serological workups can be reduced by having stored dithiothreitol red blood cells with this proving to be a useful reagent for investigating anti-CD38.

Transforming the transfusion process in myelodysplastic syndromes (MDS) to improve quality of life: an Australian-led international pilot trial of weekly personalised matched red blood cell (RBC) transfusion

Mo A[1,2,3], Stanworth S[4,5,6], Wood E[1,2], Shortt J[2,7], Charlton A[6,8], Evers D[9], Hoeks M[9], Pritchard E[10], Daly J[11], Hodgson C[12,13,14,15], Opat S[2], Bowen D[16], Aoki N[1], St George G[1], Fox V[1], Reynolds J[17,18], Le T[1], McQuilten Z[1,2]

[1]Transfusion Research Unit, Monash University, Melbourne, Australia, [2]Monash Haematology, Clayton, Australia, [3]Austin Pathology, Heidelberg, Australia, [4]Oxford University Hospitals, Oxford, United Kingdom, [5]University of Oxford, Oxford, United Kingdom, [6]NHS Blood and Transplant, London, United Kingdom, [7]School of Clinical Sciences, Monash University, Melbourne, Australia, [8]Department of Haematology, The Newcastle Upon Tyne NHS Foundation Trust, Newcastle, United Kingdom, [9]Department of Haematology, Radboudumc, Nijmegen, Netherlands, [10]School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia, [11]Australia Red Cross Lifeblood, Brisband, Australia, [12]The Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, Australia, [13]The Alfred, Melbourne, Australia, [14]The George Institute for Global Health, Sydney, Australia, [15]University of Melbourne, Melbourne, Australia, [16]Department of Health Sciences, University of York, York, United Kingdom, [17]Department of Haematology, The Alfred, Melbourne, Australia, [18]Australian Centre for Blood Diseases, Monash University, Melbourne, Australia

Aim: Anaemia and transfusion-dependence is common in MDS and associated with poorer quality of life (QoL). Current practices of regularly transfusing multiple RBC units every few weeks may result in peaks and troughs in haemoglobin (Hb), yet observational data suggests QoL may be improved by maintaining a more stable Hb. The REDDS2 (Red Cell Transfusion in MDS) trial is investigating the feasibility of a weekly personalised low-dose RBC transfusion schedule in transfusion-dependent MDS patients to maintain more stable Hb, and the effects of this on QoL and physical function.

Method: Using an n-of-1 trial design, each patient receives 2 treatment arms, with randomly allocated sequence: arm A (patient's usual transfusion schedule) and arm B (weekly transfusion). Transfusion algorithms are individualised. To facilitate timely delivery of weekly transfusion and reduce delays in arm B, extended-matched RBCs (D,c,C,E,K,Fya,Jka,Kpa) are provided and patients are transfused based on the previous week's Hb and antibody screen. Primary outcome is feasibility of delivering weekly transfusion. Secondary outcomes: RBC usage, QoL, functional activity measures, adverse events (including alloimmunisation). A qualitative sub-study explores patient and staff experiences of weekly versus standard transfusion. Target sample size is 30 patients.

Results: REDDS2 is open at 8 sites in Australia, Netherlands and UK. Recruitment commenced in 2020 and was delayed due to COVID19. Eight patients have completed treatment. No serious adverse events have been recorded. The trial is ongoing. Inter-country differences in patient testing and provision of RBCs on trial have been observed (table 1).

Table 1: Inter-country differences in provision of RBCs

	Australia	England	Netherlands
Arm A (standard of care)	ABO Rh(D) compatible	ABO Rh(D) compatible	ABO Rh(D) compatible
Arm B: patient testing to select matched units	Genotype or serological phenotype (depends on local availability and practice)	Genotype or serological phenotype (depends on local availability and practice)	Serological phenotyping
Arm B: additional requirements	Donor units additionally require Kpa phenotyping by hospital blood bank as this is not routinely provided by national blood supplier	Donor units additionally require Kpa phenotyping by hospital blood bank as this is not routinely provided by national blood supplier	Nil

Conclusion: Transfusion has been integral to MDS management for many years, yet the efficacy of current transfusion arrangements has never been tested in a clinical trial. REDDS2 seeks to improve the transfusion pathway and optimise transfusion outcomes. Results will inform the design of a future definitive trial.

Insights into the usage of immunoglobulin for haematological medical conditions in Australia

Morosin V[1]
[1]*National Blood Authority, Canberra, Australia*

Aim:

Immunoglobulin (Ig) treatment offers significant therapeutic benefit for many haematology patients. An analysis of Ig usage data was undertaken to provide insight into how Ig is used to treat haematological medical conditions in Australia.

Method:

Analysis of data collected through National Blood Authority (NBA) systems from 2012-13 to 2021-22.

Results:

For over ten years, the total demand for Ig in Australia has grown by more than 10% annually, peaking at 12.4% in 2015-16. However, since 2018-19, there has been a significant reduction in the annual rates of increase – see Table 1.

Table 1. Growth in Ig usage (year on year) 2012-13 to 2021-22.

2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
10.7%	11.0%	10.2%	12.4%	11.2%	10.6%	7.2%	6.7%	7.4%	6.9%

In 2021-22, Australian governments supported 23,187 patients to access Ig products at a cost of $810.4 million.[4] Haematology patients accounted for 28% of total Ig usage (9,746 patients) and approximately 33% of the patients accessing SCIg (743 patients out of 2,217 total SCIg patients).

Non-Hodgkin lymphoma, multiple myeloma, and chronic lymphocytic leukaemia were identified as the top 3 haematology conditions for which Ig treatment was used,[5] and the analysis showed that the number of patients using Ig for these conditions increased by 94% between 2012-13 and 2021-22. A report developed for the Leukaemia Foundation in 2019 showed that the incidence of leukaemia, lymphoma and myeloma has increased by more than 80 per cent over the past 20 years.[6]

Conclusion

Although the overall growth in Ig usage has significantly slowed since 2018-19, the demand for Ig treatment for haematological patients has increased at a much faster rate than the rate of population increase.

[4] Cost includes cost of plasma for fractionation
[5] These specific conditions fall under the *Acquired hypogammaglobulinaemia* medical condition
[6]Insight Economics 2019, *State of the Nation: Blood Cancer in Australia*, accessed 26 May 2023 State of the Nation: Blood Cancers in Australia - Leukaemia Foundation. This report is based on AIHW data.

Are you positive you want negative?

Orr A[1], Maxwell E[1]
[1]*Melbourne Pathology Services, Collingwood , Australia*

Aim: 16% of the Australian population are RhD negative and 6.5% are O RhD negative[(1)] but group O RhD negative represents up to 17% of issues to AHPs[(2)]. The national statement for the Emergency Use of Group O Red Blood Cells[(2)] recommends limiting issue of emergency O RhD negative to women ≤50 yo and males ≤18 yo. We sought to understand current usage of our emergency O RhD negative inventory, to inform the impact of a change in practice reflecting the new statement.

Method: Retrospective audit of emergency O RhD negative inventory for 2021 & 2022, held in 6 x satellite fridges & 4 x 24/7 laboratories. Parameters assessed included frequency of utilisation, recipient demographics, blood group/screen & adverse events.

Results: 1145 units designated emergency inventory: 428 units obstetric sites.
157 units (14%) transfused to 99 patients (1-4 units):17% obstetric sites.
M:F 0.8. Median age 65 (0-95): Females ≤50 (18%), Males ≤18 (1 unit, neonatal).
Valid G&S at transfusion in 11 pts: 3 pts RhD negative (4 units), including 2 pts O RhD negative (3 units).
All blood groups : 11 pts RhD negative (18 units), including 7 pts O RhD negative (12 units)
Ab screen: 1 x current autoAb and anti-M, 1 x historic autoAb, 1 x current anti-K, 1 x historic anti-K.
35% of recipients no subsequent serologic investigations.
No patient with a transfusion related event.

Conclusion: A significant number of units are quarantined as inventory (63% non-maternity sites), with only a small proportion transfused, and 89% issued to RhD positive patients. Conversion of O RhD negative to O RhD positive (obstetric excluded), would release 717 units with no safety issues identified. Findings support a change of practice would be both effective and tolerable. Local hospital education will be required for implementation.

References

- Hirani, R., Weinert, N. and Irving, D.O. (2022) 'The distribution of ABO RHD blood groups in Australia, based on blood donor and Blood Sample Pathology Data', Medical Journal of Australia, 216(6), pp. 291–295. doi:10.5694/mja2.51429.

1) NBA (2023) Group O negative red blood cell management| National Blood Authority. Available at: https://www.blood.gov.au/group-o-negative-red-blood-cell-management

Australian Haemochromatosis Patient Register

Prince D[1]
[1]*Haemochromatosis Australia, Meridan Plains, Australia*

Aims of the register: Haemochromatosis Australia received a Department of Health and Aged Care grant to build a national cloud-based haemochromatosis patient register, with the aims of

- supporting research into the role of iron in a number of related chronic health conditions including liver disease, arthritis, diabetes and heart conditions, and future research into the impact of iron on the brain
1. contributing to improved health outcomes for people diagnosed with haemochromatosis, and
2. supporting research on innovative therapies to treat haemochromatosis

Why is a register important? While individual clinicians in different specialty areas (haematologists, rheumatologists, gastroenterologists, hepatologists, cardiologists, endocrinologists and pathologists) maintain patient information in their own systems until now there has been no national registry, enabling the collection of patient information *across* specialty areas that deal with the treatment and outcomes of haemochromatosis.

Collaboration partners: The register is a collaborative initiative of Haemochromatosis Australia in partnership with QIMR Berghofer Medical Research Institute, Edith Cowan University (ECU), the Queensland University of Technology (QUT) and the Hunter Medical Research Institute (HMRI) with support from Australian Red Cross Lifeblood. Consultations with patients, clinicians and researchers have contributed to the register design.

Promoting the register: Haemochromatosis Australia identified Blood 2023 as a key event for informing haematologists, pathologists and venesection nurses about the register and to seek their support in enabling collection of patient information.

The poster will illustrate
- register structure
- information interfaces
- patient registration process
- clinician and researcher access

Haemochromatosis Australia (www.ha.org.au) provides the following:
- a telephone information and support line (1300 019 028)
- booklets and information sheets for patients and health professionals downloadable for free
- the My Iron Manager app launched in 2019 (enabling patients to track their iron studies results and locate venesection providers around Australia by postcode or town)

Genotypic and phenotypic comparison of HNA-3 in sheep

Radenkovic F[1,3], Burton M[2], Hassell P[2], Chiaretti S[1], Bui X[3], Jones M[3], Flower R[1], Tung J[1,4,5]

[1]Stategy and Growth, Australian Red Cross Lifeblood, Brisbane, Australia, [2]Pathology and Clinical Governance, Australian Red Cross Lifeblood, Brisbane, Australia, [3]Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane, Australia, [4]Faculty of Medicine, University of Queensland, Brisbane, Australia, [5]Faculty of Health, Queensland University of Technology, Brisbane, Australia

Aim: Transfusion-related acute lung injury (TRALI) is a potentially life-threatening adverse transfusion reaction. Many severe cases of TRALI relate to transfusion of antibodies against human neutrophil antigen (HNA) 3. Characterization of the HNA-3 antigen on choline transporter-like protein 2 (CTL2) led to the discovery that human anti-HNA-3a alloantibodies show some cross-reactivity with mouse CTL2. This suggested that human anti-HNA-3a antibodies may react with CTL2 from other species. Sheep have previously been used to model non-antibody mediated TRALI and were therefore assessed for their potential to model for anti-HNA-3a mediated TRALI.

Method: Sequences for CTL2 and the gene that encodes it (SLC44A2) from humans, mice, and sheep were compared using data from publicly available gene datasets on Ensembl genome browser. Corresponding protein analysis was performed using Universal Protein Resource (UniProt), a comprehensive resource for protein sequence and annotation data. Clustal 2X was used to group and colour-code each amino acid residue based on their hydrophobicity and electrostatic potential in order to compare physical properties of amino acid sequence of CTL2 between species. Following in silico comparison, binding experiments were performed using flow cytometry with human serum containing an anti-HNA-3a antibody to human, mouse and sheep cells.

Results: Although sheep SLC44A2 and CTL2 shared high homology with both human (90% and 95%, respectively) and mouse (89% and 95%, respectively) counterparts, including the Arg154 residue responsible the HNA-3a variant, no significant anti-HNA-3a alloantibody binding was observed with sheep cells.

Conclusion: This study suggests that sheep would not be a suitable large animal in which to model TRALI mediated by human anti-HNA-3a antibodies. Although the single nucleotide polymorphism responsible for the HNA-3 polymorphism is known, further work is required to understand the epitope of anti-HNA-3a alloantibodies.

Anti-N antibody mimicking an antibody to a high incidence antigen in a U-negative patient

Revoltar M[1], Lopez G[2], Clarke L[2]
[1]Royal North Shore Hospital, St Leonards, Australia, [2]Australian Red Cross Lifeblood, Alexandria, Australia

Background: Anti-N antibodies are usually IgM antibodies inactive above 25°C and not clinically significant.[1] The N antigen is present on the transmembrane glycophorin A.[2] A truncated homologous sequence is also present on glycophorin B (GPB), referred to as the 'N' antigen. U-negative individuals do not express GPB, and therefore lack the 'N' antigen. A clinically significant anti-N antibody with specificity to both N and 'N' antigens can form in individuals negative for N and U antigens.[3,4] We report a case of an anti-N antibody in an N–U– patient mimicking an antibody to a high incidence antigen.

Case presentation: A 29-year-old male of African background presented with lacerations to the face and neck following a chainsaw injury. He denied previous transfusions. Admission haemoglobin was 135 g/L. His blood group was B, D+C–E–c+e+, K–, Fy(a–b–), Jk(a+b+), M+N–S–s–. The 3-cell screening panel and 11-cell identification panel was pan-reactive by indirect antiglobulin test but the auto control was negative, suggestive of an antibody against a high incidence antigen. Samples were sent to Australian Red Cross Lifeblood for further investigation.

Results: Serological testing showed reactions were not destroyed by DTT or trypsin but were abolished after treatment with papain. This pattern excluded an anti-U antibody. An anti-N antibody was identified and confirmed by reactions against M–N+U+ and M–N+U– cells but negative against N–U– and M^kM^k (M–N–S–s–) cells. Genotyping predicted M+N–S–s–U–, Fy(a–b–), heterozygosity for weak D type 4.0 or 4.3, and VS+V+.

Conclusion: The patient underwent surgical repair of the deep neck lacerations. His post-operative haemoglobin was 125 g/L and he did not require blood transfusion. Autologous donations and family phenotyping studies will be pursued.

References:

1. Kumawat V, Jain A, Marwaha N, Sharma RR. Anti-N antibody reacting at 37°C: An unusual occurrence interfering with routine testing: Two interesting cases. Asian J Transfus Sci 2015;**9**: 92-3.
2. Daniels G. Human blood groups. 3rd edition. Oxford: Wiley-Blackwell Publishing, 2013.
3. Ballas SK, Dignam C, Harris M, Marcolina MJ. A clinically significant anti-N in a patient whose red cells were negative for N and U antigens. Transfusion 1985;**25**: 377-80.
4. Yoell JH. Immune anti-N agglutinin in human serum. Report of apparent associated hemolytic reaction. Transfusion 1966;**6**: 592-3.

Characterisation of an anti-SARS-CoV-2 hyperimmune immunoglobulin prepared from COVID-19 convalescent plasma produced by Tangential Flow Electrophoresis

Rozen L[1], **Mahler S[1,2]**, Saputro B[1], Maoudis C[1], Turville S[3], Kelleher A[3], Nair H[1,2]
[1]Aegros, Macquarie Park, Australia, [2]Australian Institute for Bioengineering and Nanotechnology, University of Queensland, St Lucia, Australia, [3]The Kirby Institute, University of NSW, Sydney, Australia

Aim: Produce an anti-SARS-CoV-2 hyperimmune immunoglobulin (hIg) meeting EP standards by Tangential Flow Electrophoresis (TFF) (HaemaFrac® technology). High titre COVID-19 convalescent plasma (CCP) taken from individuals who have recovered from COVID-19 or hIg produced from CCP can be administered for prophylaxis against infection and /or potential treatment of disease resulting from the infection.[viii]

Method: Pooled CCP was fractionated (Aegros Ltd, Sydney) using HaemaFrac® technology, to produce an Aegros hIg against COVID-19 and characterised according to established testing procedures. HaemaFrac® technology is based on electro-separation of plasma proteins according to their charge and/or size in aqueous solution, maintaining proteins in their soluble, native state.

Results: Extensive characterisation demonstrates production of hIg complying with European Pharmacopoeia (EP) for human normal Ig for intravenous administration, and with features that differentiate the product due to the mild conditions of production. HaemaFrac® produced an anti-SARS-CoV-2 hIg which closely reflected source plasma IgG subclass proportions, importantly conserving the IgG3 subclass. Anti-SARS-CoV-2 spike IgG levels were concentrated around 9-fold from pooled CCP for manufacture, while viral neutralisation was concentrated up to 6-fold against ancestral Wuhan strain, as well as variants of concern Beta, Delta and even Omicron BA.2 and BA.5. A battery of analytical techniques including HPLC, SDS-PAGE, mass spectrometry (LC-MS-MS) and ELISA were used for full product characterisation.

Conclusion: The biomanufacturing of the Aegros hIg against COVID-19 by HaemaFrac® technology produces an hIg preparation enriched in anti-SARS-CoV-2 IgG. The clinical utility of the Aegros hIg against COVID-19 as a passive immunity modality is currently being evaluated in a phase 1/2 clinical trial.[ix]

References:

When Guidelines Fail – Alloimmunisation to the RhD Antigen due to Platelet Transfusion

Rushford K[1], Ellison A[1], Bennett A[1], Wood E[1]
[1]*Monash Medical Centre, Melbourne, Australia*

Introduction: Donor platelets contain a small but variable number of red cells, so the ANZSBT Guidelines recommend transfusing RhD Negative platelets wherever possible in RhD negative females of childbearing potential, and that RhD Immunoglobulin (RhD-Ig) should be offered if RhD Positive platelets are transfused. We report a case where a patient received multiple units of RhD Ppositive platelets in the setting of massive transfusion, where RhD prophylaxis failed to prevent alloimmunisation.

Case Report: A young woman presented with palpitations and tachycardia. She has a complex medical history including an underlying inherited disorder (but no immunodeficiency). Her condition deteriorated with the development of cardiogenic shock with metabolic acidosis and hyperlactataemia. Emergency cardiac surgery was performed requiring a complex and extensive procedure of 3.5 hours duration. The pretransfusion testing was Group O RhD Negative with no antibodies detected.

The massive transfusion protocol was activated, and the patient received multiple blood products during the initial surgery. Ongoing bleeding and haemodynamic instability required a return to theatre. She was coagulopathic with multiorgan failure. During the initial surgery and over the next four days the patient received a total of 14 units of red cells, 18 FFP, 12 platelets, 100 cryoprecipitate and 7000 IU of Prothrombinex-VF. Supply issues of RhD negative platelets necessitated the infusion of six units of RhD Positive platelets (3 pooled and 3 apheresis). 250 IU of RhD-Ig was administered IM after the first infusion of D Positive platelets.

The antibody screen was negative after the IM RhD-Ig. Another antibody screen collected 18 days later demonstrated anti-D, and alloimmunisation was inferred. Further follow up testing is planned.

Conclusion: The recommended dosage of RhD-Ig was ineffective at preventing alloimmunisation in this immunocompetent patient who received multiple RhD Positive platelets. The Australian Red Cross LifeBlood Blood Component Information states that one 250IU dose provides cover for six weeks of platelet transfusions. Given the adverse outcome in this case, further guidance may be helpful in guiding practice in the setting of multiple transfusions of RhD Positive platelets.

Can serological testing be reduced in favour of matched phenotyped red blood cells in patients with warm autoantibodies?

Smallman C[1]
[1]*SA Pathology, Adelaide , Australia*

Aim: To determine if decreasing adsorption frequency and the transfusion of phenotype matched RBCs in patients with Warm autoantibodies (WAA) and warm autoimmune haemolytic anaemia (WAIHA) improves the time the RBCs are available, without increasing alloimmunisation rates or transfusion adverse events.

Method: Review of current literature and various Transfusion Associations' publications for changes in practice for the investigation and transfusion management of patients with WAA and WAIHA, focusing on provision and transfusion of phenotype matched RBCs and the safety of decreasing the frequency of alloadsorptions.

Patient records were reviewed from 2020 to 2021 from 17 public South Australian blood banks with a total of 441 samples collected from 132 patients.

Results: Following introduction of the prophylactic antigen matched (PAM) protocol (2020-2021), 163 alloadsorptions were omitted saving 412 hours and serological indirect agglutination test (IAT) crossmatching was omitted for 150 donor units saving 41.5 hours. In total 454 hours of pretransfusion testing was omitted improving the time RBCs were made available to patients. Alloimmunisation was detected in two patients, however this is not believed to be attributed to the protocol, and no adverse events were observed.

Conclusion: It is recommended that the patient's complete phenotype be determined during their initial pretransfusion testing either by molecular techniques or in combination with serological phenotyping to allow the use of PAM donor units.
By applying the PAM protocol patient benefits include a decrease in alloimmunisation rates and the risk of DHTR. With the PAM protocol the costly, lengthy, and consuming pretransfusion adsorption studies would be limited, to being performed monthly while the WAA is active and ensuring readily available compatible red cells for transfusion.
Further larger studies are required to confirm that the PAM algorithm is efficient, cost effective and safe.

Applications of data mining at New Zealand Blood Service

So R[1]
[1]*New Zealand Blood Service, Auckland, New Zealand*

Aim: To give insight into how data mining can be applied at the Blood Services to verify science or to generate new science.

Method:

Locale: New Zealand Blood Service, Auckland.

Sample number: n=1757

Principal test: Fibrinogen testing

Statistical analysis employed: Histogram, moving average, Pearson's Coefficient of Skewness

- Take a raw data set (i.e. results from cryoprecipitate products) and through data cleansing (removal of erroneous/incomplete data) and selection (target parameter of interest and relevancy), generate the target data.
- Take the target data and perform pre-process work (i.e. limiting one result to each donor to reduce the over-representation of frequent donors)
- Using the processed data, find ways to transform it and for pattern identification to generate knowledge.

Results: According to the literature, fibrinogen concentration has a non-normal distribution in the human population as a subgroup of the population has mutations in promoter to the β-fibrinogen gene (-455G/A and -854G/A), leading to over-expression of the β chain. Since the β chain is the rate-limiting step in the production of the mature fibrinogen molecule, the hypothesis is that the fibrinogen concentration of the cryoprecipitate product would have a non-normal distribution.

Results (after data mining): Shows a bimodal distribution for the fibrinogen concentration in the cryoprecipitate product, however, the fibrinogen concentration of our plasma donors has a normal distribution.

Conclusion: According to the literature, the rare alleles of -455G/A and -854G/A polymorphisms are associated with significant plasma fibrinogen levels in healthy middle-aged men (approximately 30% of the population). Factors such as age, body mass index and smoking are important environmental factors associated with fibrinogen concentration but were not available and therefore were not considered. Further analysis is required to comprehend the results observed. If this subgroup does exist in the population, blood services could aim to identify and recruit this subgroup to their cryoprecipitate donor panel.

Incidence of RhD-Negative patients receiving RhD-Positive red blood cell units in massive transfusion varies across Australia: Data from the Australian and New Zealand Massive Transfusion Registry (ANZ-MTR)

Sparrow R[1], Haysom H[1], Tacey M[2], Wellard C[1], Waters N[1], McQuilten Z[1], Wood E[1]
[1]Transfusion Research Unit, Monash University, Melbourne, Australia, [2]Centre for Health, Education and Research, Northern Health, Melbourne, Australia

Aim: Supply of RhD-matched RBCs for RhD-Negative patients requiring massive transfusion (MT) may become exhausted. We examined the incidence of RhD-mismatched transfusion (defined as ≥1 unit RhD-Positive RBCs transfused to a RhD-Negative patient) using ANZ-MTR data.

Method: MT was defined as ≥5 RBC units transfused in 4h. Patient demographics, hospital admission and transfusion records were available for 1,207 MT-episodes in adult RhD-Negative patients between 2011-2018, representing 13.4% of all MT-episodes (n=9,013). Descriptive statistics were performed.

Results: Of 1,207 RhD-Negative MT-episodes, 268 (22.2%) were RhD-mismatched and occurred across 23/28 participating hospitals, with wide difference in incidence between hospitals (1.5-63.5%). To identify possible explanations, hospitals with incidence ≤20% or ≤20 RhD-Negative MT-episodes were coded 'Set-1' sites (n=18); those with incidence >20% were coded 'Set-2' sites (n=5). While 33.2% (89/268) RhD-mismatched cases occurred across the 18 Set-1 sites, 66.8% (179/268) occurred across only five Set-2 sites, equating to incidence rates of 10.6% for Set-1 and 49% for Set-2. In-hospital mortality, hospital length-of-stay post-MT, proportion of MT-start after-hours, ABO frequencies and sex were not significantly different between Set-1 and Set-2.

More Set-2 MTs started later than 2h after hospital admission (72.6% versus 56.2%; p=0.007). Set-2 cases received fewer RBC units within 24h-post MT-start (median [IQR]: 10 [7-14 versus 14 [9-20] units; p<0.001), but more of those were RhD-Positive units (53% versus 36%; p<0.001). More Set-2 cases received RhD-Positive RBCs at MT-start (29% versus 9%; p=0.0002), with 20 cases at Set-2 sites transfused RhD-Positive RBCs exclusively. Set-2 cases were younger (mean 60±18years versus 66±15years; p=0.013); and more were associated with cardiothoracic surgery (29.1% versus 12.4%; p=0.002).

Conclusion: Overall, 22% of RhD-Negative patients received ≥1 RhD-Positive RBC unit during their MT-episode; however, RhD-mismatch incidence rate was significantly higher at some sites. Follow-up to understand clinical consequences is warranted.

High mobility group box 1 (HMGB1) has minimal immunomodulatory effects in models of transfusion

Tung J[1,2,3,4], Tran T[1], Maas H[1], Chong F[1], Dean M[1,2,4]

[1]Australian Red Cross Lifeblood, Brisbane, Australia, [2]University of the Sunshine Coast, Moreton Bay, Australia, [3]University of Queensland, Brisbane, Australia, [4]Queensland University of Technology, Brisbane, Australia

Aim: During routine storage of platelet concentrates (PCs), activated platelets release the immunomodulatory protein, high mobility group box 1 (HMGB1). This study aimed to investigate if concentrations of HMGB1 found in PCs (1-100ng/mL) exerted immunomodulatory effects within laboratory models.

Method: To model transfusion, whole blood from volunteers (n=10) was left untreated or treated with recombinant HMGB1 (rHMGB1) at 1, 10, 100 and 1,000ng/mL, and cultured for 5.5 hours (37°C, 5% CO_2). In parallel, samples of whole blood were also treated with either lipopolysaccharide (1µg/mL) or poly I:C (50µg/mL) to model bacterial or viral infection respectively. From all three models, culture supernatant was harvested and frozen for analysis. Cytometric bead array was used to quantify IFN-α, IFN-γ, IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12p70, IP-10, MCP-1, and TNF-α in the culture supernatants. Data normality was assessed by Shapiro-Wilk testing and subsequent analysis was performed using ANOVA or Friedman's test (normally distributed or non-normally distributed data respectively, $P<0.05$ considered significant).

Results: In the whole blood model of transfusion, rHMGB1 only increased overall inflammatory responses at the 1,000ng/mL concentration (increased IL-1β, IL-6, IP-10 (all ($P<0.01$), IL-8 and TNF-α (both $P<0.05$)). In the whole blood, viral infection (poly I:C), and transfusion model, rHMGB1 only exerted pro-inflammatory effects at the 1,000ng/mL concentration (increased IL-6, IL-8 (both $P<0.0001$), TNF-α ($P<0.01$), and IL-1β ($P<0.05$). In the whole blood, bacterial infection (lipopolysaccharide) + transfusion model, rHMGB1 moderately suppressed LPS-mediated inflammation: 10ng/mL rHMGB1 suppressed IL-8 ($P<0.05$), while 1,000ng/mL rHMGB1 suppressed IP-10 ($P<0.01$) and MCP-1 ($P<0.05$).

Conclusion: In these models, at concentrations comparable to those found in PCs during routine storage, rHMGB1 exerted minimal effects on the overall inflammatory response in whole blood. This suggests that HMGB1 is unlikely to contribute to an overall inflammatory response post-transfusion; however, its impact on the function of specific immune cell subsets (e.g. dendritic cells) remains to be explored.

Novel mutation causes HPA-1 genotyping discrepancy in FNAIT

Tyler D[1], Burton M[1], Hassell P[1], Mahon D[1], Devikashri N[1], Pahn G[1]
[1]*The Australian Red Cross Lifeblood, Brisbane, Australia*

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is a complication of pregnancy when maternal IgG specific platelet antibodies cross the placenta and bind to paternally inherited platelet antigens on foetal platelets. FNAIT can result in critical neonatal thrombocytopenia, and in severe cases foetal intracranial haemorrhage and death. A standard FNAIT investigation in Australia typically includes human platelet antigen (HPA) genotyping at the HPA-1, 2, 3, 4, 5 and 15 loci on maternal, paternal and foetal/neonate samples. Here we report on two unrelated FNAIT cases, where the routine HPA genotyping method did not produce the expected result.

An in-house in vitro diagnostic (IVD) TaqMan Real-Time PCR assay (RT-PCR) is the routine HPA genotyping assay. Each FNAIT mother genotyped as HPA-1bb. Both partners were HPA-1a positive. Each neonate genotyped as HPA-1aa. The familial genotyping discrepancy indicated either a laboratory error or the presence of an undetected mutation. Repeat testing of neonate samples by RT-PCR and secondary sequence specific primer PCR (SSP) methods showed a discrepancy between results. In both cases, the SSP method gave the expected result of HPA-1ab, while RT-PCR confirmed the original result of HPA-1aa. Next generation sequencing (NGS) was performed to identify the suspected mutation.

In both neonate samples a single nucleotide substitution (SNP) (c.197T>G) leading to a missense mutation (p.L66R) was detected within the maternally inherited HPA-1b encoding ITGB3 allele. The RT-PCR assay parameters indicated that the c.197T>G SNP falls within the current sense primer location for the HPA-1 amplicon, leading to an incorrect genotype. Using a HPA genome database for known HPA-1 SNPs, the sense primer was shifted upstream by 19bp to a region with no known SNPs.

These two FNAIT cases have driven a change in the primer design of our in-house HPA-1 RT-PCR assay to ensure a more reliable technique.

National guidance for the management of red blood cell inventory

Wall L[1], Cochrane S[1], Roxby D[2]
[1]National Blood Authority, Canberra, Australia, [2]Flinders University, Belford Park, Australia

Aim: The national use of group O RhD negative RBC has increased to unsustainable levels over the last few years. The National Blood Authority (NBA) and an expert working group have developed *National Guidance for the Management of Red Blood Cell Inventory* (RBC Inventory Guidance) to accompany the recently released the joint *National Statement for the Emergency Use of Group O Red Blood Cells* (National Statement).

Method and Results: The *RBC Inventory Guidanc*e provides advice for Australian Health Providers (AHP) to assist with reviewing their RBC inventory to reduce reliance on group O RhD negative RBC and maintain appropriate ABO and RhD blood group stock numbers that meet clinical need while at a low enough level to minimise time expiry.

The *RBC Inventory Guidance* includes:

- guidance on inventory management
  - an Inventory Review Report that AHPs will be able to run through BloodNet. The report will provide five six-month periods of red blood cell data to assist AHPs with estimating their required inventory using days cover for each ABO and RhD group based on transfusion or issue patterns and the Australian population ABO and RhD groups
  - advice for AHPs located on using the Inventory Review Report, particularly for those located in regional and remote areas
  - advice on implementing inventory changes

The *RBC Inventory Guidance* complements the *National Statement*, supporting AHPs to implement the *National Statement* and reduce the national burden on group O RhD negative RBC.

Conclusion: The *RBC Inventory Guidance* supports the *National Blood Product Management Improvement Strategy 2018-2024*. Developing guidance with an expert working group allows the NBA to work in partnership with the Australian health sector and jurisdictional governments. The collaborative approach is key to improving implementation and achieving viable and ongoing results.

Investigation of microRNAs in cryopreserved platelets

Waters L[1], Marks D[1,2], Johnson L[1]
[1]*Australian Red Cross Lifeblood, Alexandria, Australia,* [2]*University of Sydney, Camperdown, Australia*

Aim: Platelets contain an abundance of microRNAs (miRNAs) which are involved in the regulation of platelet function. Alterations to the expression of miRNAs has been linked to the platelet storage lesion and apoptosis during room-temperature storage. However, changes in expression of miRNAs in cryopreserved platelets have not been examined. The aim of this study was to characterise differences in the relative abundance of apoptosis-related miRNAs in fresh and cryopreserved platelets to provide new insight into the regulation of platelet function during component storage.

Methods: Apheresis platelets (day 1 post-collection) were cryopreserved at -80°C with 5-6% dimethylsulfoxide. Cryopreserved platelets were thawed and resuspended in a unit of plasma before sampling. Samples were taken before (fresh) and after cryopreservation (n=3). RNA was extracted, reverse transcribed and PCR was performed using a miRCURY LNA miRNA Human Apoptosis Focus V2 panel (QIAGEN). The abundance of miRNA was compared between fresh and cryopreserved groups using a paired t-test. A p-value of less than 0.05 and a fold-change of >1.5 were set as cut-offs for up-regulated targets.

Results: A total of 84 apoptosis-related miRNA targets were screened. Of these, the abundance of six targets was statistically different and at least 1.5-fold higher in the cryopreserved platelets (Table 1).

Table 1: miRNAs with a relative increase in abundance in cryopreserved platelets compared to fresh platelets.

miRNA	p-value
miR-181c-5p	0.022
miR-29a-3p	0.005
miR-29c-3p	0.004
miR-32-5p	0.032
miR-365a-3p	0.047
miR-497-5p	0.018

Conclusion: Six miRNAs were differentially expressed in fresh and cryopreserved platelets. The role of these miRNAs has been documented across various cell types and disease states, providing an indication of their potential role in platelets, which likely includes the regulation of pro-survival proteins, such as Bcl-2, and pro-apoptotic proteins, such as Bak and Bax. Further research is required to fully elucidate their role in cryopreserved platelets.

Pilot of home administered subcutaneous immunoglobulin therapy in Western Australia, 2021-2022

Webster B[1]
[1]*Western Australian Department of Health, Perth, Australia*

Background: Subcutaneous immunoglobulin (SCIg) is a fractionated blood product available in Australia for treatment of a range of immunodeficiency and inflammatory conditions.[1] SCIg therapy has demonstrated benefits over intravenous immunoglobulin (IVIg) therapy including ease of administration, more stable dosing, reduced demand on health service resources and the ability to self-administer in the home.[2] Despite the identified benefits, patient access to SCIg therapy in Western Australia (WA) has been limited.

Aim: To develop a SCIg pilot program for patients receiving hospital based IVIg therapy at Royal Perth Hospital (RPH) to transition to home based SCIg therapy.

Method: Eligible haematology and neurology patients receiving IVIg therapy were recruited to the pilot from April to December 2021. A clinical nurse specialist provided training, support and continuity of care to patients. Patient surveys were conducted at baseline, midway and at completion of the pilot. Staff surveys were conducted in January 2022 following completion of the pilot.

Results: A total of 15 patients participated in the program. Surveyed patients reported a range of benefits including general health improvement, greater control of their treatment, reduced impact on lifestyle and reduced financial burden.

93% (n=15) of enrolled patients continued with SCIg therapy at the conclusion of the pilot. One patient withdrew due to minor skin reactions. Over the course of the pilot, there was no reported deterioration of patient condition, increase in acute hospital admissions or significant adverse reactions.

Staff surveyed reported that the program offered patients greater control of their health, reduced demand for hospital resources and reflected an interdisciplinary approach to patient care.

Conclusion: Self-administration of SCIg in the home is an effective alternative to hospital based IVIg therapy demonstrating benefits to patients, hospitals and staff. Evaluation of the RPH pilot program will be used to develop a framework to support the establishment of hospital based SCIg programs in WA.

References:

- Self-administration National Blood Authority Australia. Criteria for the Clinical Use of Immunoglobulin within Australia. 2018. National Blood Authority, Canberra. Accessed 01/10/2021. https://www.criteria.blood.gov.au/
1. Overton, P, M., Shalet,,N., Somers, F. et al. Patient Preferences for Subcutaneous versus Intravenous Administration of Treatment for Chronic Immune System Disorders: A Systematic Review. Patient preference and adherence. 2021;15:811. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8064718/

Improving utilization of subcutaneous immunoglobulin therapy in Western Australia through the development of a framework for hospital-based SCIg programs

Webster B[1]
[1]*Western Australian Department of Health, Perth, Australia*

Background: Subcutaneous immunoglobulin (SCIg) is a fractionated blood product available in Australia for treatment of a range of immunodeficiency and inflammatory conditions.[1] SCIg therapy has benefits over intravenous immunoglobulin (IVIg) therapy including ease of administration, more stable dosing, reduced demand on health service resources and the ability to self-administer in the home.[2] Despite the benefits of SCIg compared to IVIg therapy, utilization of SCIg therapy in Western Australia (WA) has remained below national trends.

Aim: To determine if a framework for development of a hospital-based SCIg program (the Framework) would improve utilization of SCIg in WA.

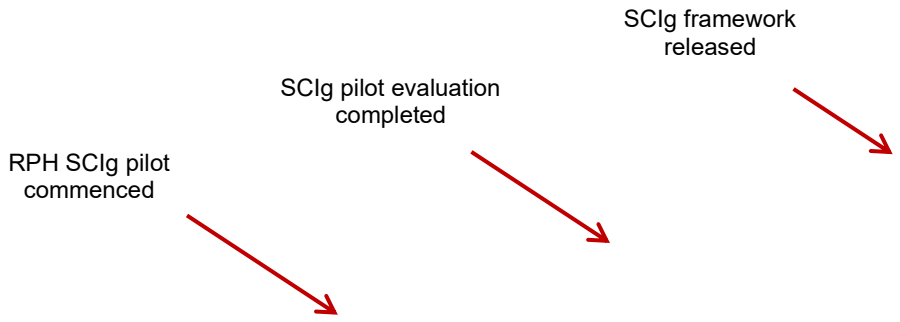
Method: A pilot program was established at Royal Perth Hospital for eligible IVIg therapy patients to transition to self-administered SCIg therapy. On completion of the pilot program, a project evaluation identified barriers to implementing hospital based SCIg programs including:

- access to sustainable activity-based funding
- provision of clinical and administrative support
- consistent patient training and support
- product dispensing arrangements

The Framework was developed in consultation with WA public health services to address identified barriers, define key roles and responsibilities and make recommendations to health services for establishment of hospital-based SCIg programs.

Analysis of WA SCIg dispensing data was used to evaluate the effectiveness of the Framework.

Results: The development and implementation of the Framework has resulted in incorporation of key recommendations into hospital-based programs and policies including the establishment of dedicated SCIg clinics, SCIg nurses and processes to sustainably fund SCIg clinic activity. The number of SCIg patients and volume of SCIg dispensed in WA has increased significantly since the start of the project and is now more closely aligned with national trends (figure 1).



Conclusion: The implementation of a state-wide SCIg framework has provided clarity and consistency in the development and delivery of hospital-based SCIg programs across WA, ensuring patients have access to appropriate and sustainably funded SCIg therapy.

Figure 1: WA SCIg patient count and volume dispensed 2018-2022

References:

2. Self-administration National Blood Authority Australia. Criteria for the Clinical Use of Immunoglobulin within Australia. 2018. National Blood Authority, Canberra. Accessed 01/10/2021. https://www.criteria.blood.gov.au/
3. Overton, P, M., Shalet,,N., Somers, F. et al. Patient Preferences for Subcutaneous versus Intravenous Administration of Treatment for Chronic Immune System Disorders: A Systematic Review. Patient preference and adherence. 2021;15:811. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8064718/

A review of delayed haemolytic and delayed serologic reactions reported to Serious Transfusion Incident Reporting program

Akers C[1], Glazebrook B[1], Bielby L[1], Davis A[2], Daly J[3]
[1]Blood Matters, West Melbourne, Australia, [2]Alfred Health, Melbourne, Australia, [3]Australian Red Cross Lifeblood, Brisbane, Australia

Introduction: Since 2017, Blood Matters Serious Transfusion Incident Reporting (STIR) program has collected delayed haemolytic (DHTR) and serologic reactions (DSTR) data.

Aim: Review incidence and associated serology of delayed reactions reported.

Method: Delayed reactions were analysed/reviewed in relation to antibodies involved and evidence of haemolysis.

Results: Financial years 2018-22, 102 validated investigations reported: 74 (72%) DSTR, 28 (27%) DHTR. All except one, red cells (RC) were the implicated component. Table 1 shows antibody history and identification. The antibody not identified in 6 reports (3 each DSTR and DHTR). Table 2 shows DHTR signs and symptoms.

Table 1. Antibody identification

	No.	Pre-existing antibody	≥2 antibodies identified post transfusion	Most common implicated antibody
DSTR	74	9 (12%)	17 (23%)	Jka 26 (35%) E 20 (27%)
DHTR	28	4 (14%)	12 (43%)	E 9 (32%) Jka 7 (25%)

Table 2. DHTR: Signs and symptoms reported (25 of 28 reports)

Jaundice / increased bilirubin	Anaemia / fall in Hb / failure to increment	Abnormal blood film	Haemoglobinuria	LDH elevated	Haptoglobin low	Positive DAT
15	21	5	2	21	19	23

DHTR: 12 reported no increase in care for patients, 11 temporary increase in care, and 6 increased length of stay; 14 required further transfusion, 3 readmitted, 12 no treatment.

Conclusion: DSTR are reported more frequently than DHTR. Although 9 DHTR resulted in a more severe outcome, usually readmission/increased stay, no patients required ICU admission or renal support.

RC antibodies may become undetectable over time and mild haemolysis may be unrecognised, explaining low rates of reporting. Alloantibody registries, e.g. in Western Australia, is an important preventative strategy to reduce risk of DHTR and associated morbidity/resource utilisation. Continued documentation of DHTR and DSTR is important to guide transfusion practice and need for preventative strategies, including to assess the impact of recent changes to recommendations for transfusion of emergency group O RC.

RFID tagging of blood products - improving cold chain compliance

Dhir V[1], Tran L, Knapman L, Motum P
[1]*NSW Health Pathology, Sydney, Australia*

Aim: To study an upgraded RFID tracking system to reduce blood and blood products waste dispensed from blood bank to operating theatres.

Method: This study collected data from a new RFID system, comparing key performance indicators from manual and previous RFID systems.

Performance indicators included:

- time savings of staff processing dispense transaction,
- comparison pre and post study blood/blood product waste
- Cost savings of any reduced blood/blood product waste.

Other improvements assessed were temperature monitoring, cold chain records, speed of scanning in operating theatre fridge and any positive ramification of faster scanning.

Results: Since implementation of the new RFID system, medical and transfusion staff have much more confidence in the cold chain compliance of blood and blood products used in theatres.

Pre-study there was 10 episodes of blood/blood product wastage from January - December 2020, 10 units of red blood cells (RBC) and 11 units of extended life plasma (ELP) were discarded due to lack of cold chain records, totalling wastage costs of $5620.

There were 2 episodes of blood products wasted during the study from January - December 2021, with zero RBC and 6 ELP units discarded, totalling wastage costs of $1116.
On average it takes 45 mins of staff hours to investigate and release products to general stock (or discard if non-compliant). There is 80% savings in staff hours when comparing the old RFID system with new RFID system.

Conclusion: The study demonstrated cost savings with blood/blood product wastage and time savings in staff hours. There were nil incidents reported involving removal of wrong blood/blood product or near misses with new RFID system. The benefits and success of this trial supports the plans for future upgrades to blood dedicated fridges in the Emergency Department and Cancer Therapy Centre.

Evaluation of the analytical performance of Immulab, Bio-Rad, Grifols and Ortho reagent blood cells in antibody screening and identification

Guglielmino J[1,2], Morris F[1], Grattidge C[2], Jackson D[1]
[1]*RMIT University, Bundoora , Australia,* [2]*Immulab Pty Ltd, Parkville, Australia*

Aim: The detection and identification of clinically significant antibodies to red cell antigens forms the foundation of safe transfusion practices. The reagent red blood cells (RRBCs) used in antibody detection and identification vary in sensitivity and specificity and should therefore be carefully considered before implemented in routine patient testing. This study aims to evaluate the diagnostic performance of RRBCs used in Australia.

Method: Over 150 patient-derived plasma samples containing clinically significant alloantibodies were tested using column agglutination technology (CAT) with Immulab, Bio-Rad, Grifols and Ortho Clinical Diagnostics screening and identification RRBCs. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for Immulab RRBCs on each platform with direct comparisons to equivalent Bio-Rad, Grifols and Ortho RRBC performance measures. Differences in reaction strength were scrutinized and the additional effort required to resolve different antibody specificities was also investigated.

Results: Immulab 0.8% RRBCs demonstrated superior sensitivities, NPVs and accuracies compared to equivalent Bio-Rad, Grifols and Ortho RRBCs in the detection of clinically significant antibodies when tested using Bio-Rad, Grifols and Ortho Diagnostics CAT respectively. Factoring in occurrence of false negative results, differences in reaction strength and antibody resolution efficiency, the 0.8% RRBCs with the overall greatest diagnostic accuracy was found to be manufactured by: Immulab > Ortho > Bio-Rad > Grifols.

Conclusion: Failure to detect weak clinically significant alloantibodies during pre-transfusion testing may increase the risk of haemolytic transfusion reactions in patients requiring treatment. Selection of RRBCs should be carefully scrutinized by any laboratory performing transfusion testing.

Patient reported outcomes of Serum Eye Drops (SED) manufactured from Australian blood donations and packaged using MEISE vials

Hirani R[1,2], Mondy P[1], Gemelli C[3], Kakkos A[3], O'Donovan J[1], Diaz P[1], Knight E[3], Irving D[1,4]
[1]Australian Red Cross Lifeblood, Sydney, Australia, [2]Macquarie University, Sydney, Australia, [3]Australian Red Cross Lifeblood, Melbourne, Australia, [4]University of Technology Sydney, Sydney, Australia

Background: Dryness and inflammation of the ocular surface can result in severe discomfort and reduced vision. Serum from blood donation can substitute for tears as it contains similar biological factors. Australian Red Cross Lifeblood manufactures autologous serum eyedrops (Auto-SED) and patient-tailored (allogeneic) SED (PT-SED). This study aimed to conduct a prospective patient-reported outcome analysis to examine efficacy of SED.

Methods: Patients that were provided SED between 1 November 2021 and 30 June 2022 were invited. Patients completed standardised health questionnaires including the dry eye questionnaire (DEQ5), health-related QOL (SF-8^{TM}), Functional Assessment of Chronic Illness Therapy (FACIT) and general wellbeing scores. Existing patients were surveyed once, and new patients were surveyed at baseline then at 3 and 6 months post-treatment.

Results: Completed surveys were obtained for 24 existing and 40 new Auto-SED patients and from 10 existing and 8 new PT-SED patients. The mean age was 57yrs (±13) for Auto-SED and 70-74yrs (± 11) for PT-SED patients. Symptom duration ranged from 6-17yrs and nearly all patients had tried other treatments. DEQ5 scores in new Auto-SED patients improved from 13.9 (±2.9) to 10.6 (±3.4) within 6 months and for PT-SED patients improved from 12.3 (±3.9) to 11.4 (±2.8). For Auto-SED patients wellbeing scores improved from 7.2 (±1.9) to 7.8 (±1.7), however for PT-SED patients decreased from 6.9 (±2.6) to 6.1 (±2.9). QOL SF-8^{TM} measures improved in Auto-SED patients from 19.5 (±6.5) to 18.7 (±6.0) but did not improve in PT-SEDs, 26.4 (±9.8) to 29.3 (±7.7). Patients used SED approximately 5 times per day with 2 drops each time.

Conclusions: SED improved dry eye symptoms in the majority of patients with positive feedback on DEQ5 measures. For patients receiving PT-SED some measures decreased during the survey period, however concurrent changes in other comorbidities were not assessed and these patients gave positive feedback for SED.

Evaluation of serum eyedrop MEISE vial packaging for Australian patients

Hirani R[1,2], Mondy P[1], Gemelli C[3], Kakkos A[3], O'Donovan J[1], Willis C[3], Diaz P[1], Knight E[3], Irving D[1,4]

[1]Australian Red Cross Lifeblood, Sydney, Australia, [2]Macquarie University, Sydney, Australia, [3]Australian Red Cross Lifeblood, Melbourne, Australia, [4]University of Technology Sydney, Sydney, Australia

Background: Dryness and inflammation of the ocular surface can result in severe discomfort and reduced vision. Serum from blood donation can substitute for tears as it contains similar biological factors. Australian Red Cross Lifeblood manufactures autologous serum eyedrops (Auto-SED) and patient-tailored (allogeneic) SED (PT-SED). In May 2020, an eye dropper vial packaging system (MEISE) was introduced to replace segmented tubing. This study assessed patient reported outcomes on the new packaging.

Methods: Patients provided with SED between 1 November 2021 and 30 June 2022 were invited. Patients were surveyed about the SED supporting materials, how they were using their drops and disposal rates. Existing SED patients were surveyed once, and new SED patients were surveyed 3 and 6 months after receiving their first treatment.

Results: Completed surveys were obtained for 24 existing and 40 new Auto-SED patients and from 10 existing and 8 new PT-SED patients. The mean age was 57yrs (±13) for Auto-SED and 70-74yrs (± 11) for PT-SED patients. More Auto-SED patients reported receiving the information brochure (73-88%) compared to PT-SED patients (45-66.7%). Disposal of damaged vials was low with 8 Auto-SED patients discarding a maximum of 7 vials and 2 PT-SED patients discarding a maximum of 3 vials. Up to 85 vials were discarded due to expiry across 17 Auto-SED patients with up to 180 vials discarded by 3 PT-SED patients. Some patients indicated vials were difficult to close once opened. It was also challenging for patients to travel with frozen vials.

Conclusions: Efficacy of SED is well documented but to collect serum and manufacture SED is costly, requiring several manual processes and alternate distribution logistics. Feedback from SED patients enables improvement of user instructions and provides information to regulators on efficacy for funding. Many patients indicate that SED are a vital, product to treat their dry eye condition.

Analysing the Prevalence of Extended Rh Blood Group Antigens Within Australian Blood Donors

Hirani R[1,2], Mondy P[1], Powley T[3], Irving D[1,4]
[1]*Australian Red Cross Lifeblood, Sydney, Australia,* [2]*Macquarie University, Sydney, Australia,* [3]*Australian Red Cross Lifeblood, Brisbane, Australia,* [4]*University of Technology Sydney, Sydney, Australia*

Background: Recently it was shown that the proportion of RhD positive and B and AB individuals has increased in alignment with the changing demographics of Australia. Contemporary data on the prevalence of other clinically relevant blood groups has not been reported. This study aimed to analyse the proportion of Rh(C, c, E, e) blood group phenotypes in Australian blood donors.

Methods: Blood group phenotype data from all blood donors who donated in 2019 were extracted from the National Blood Management System (NBMS) administered by Australian Red Cross Lifeblood. Testing for Rh(C, c, E, e) and K blood group phenotypes has been performed on all first-time donors using the PK7300 Automated Microplate System (Beckman Coulter) since 2006.

Results: Data from 477,602 blood donors, including 103,779 (21.7%) first time donors, was analysed. Previous estimates of the R_1R_1 (D+C+E-c-e+) phenotype was 17.3%. After analysing the 2019 blood donor data, we found this had increased to 20.6% and 24.0% in first-time blood donors. Previous estimates of the rr (D-C-E-c+e+) phenotype was 16.4%. After analysing the 2019 blood donor data, we found that the prevalence was 19.8% in the total blood donor panel but was lower (15.2%) in first-time blood donors. There is a higher proportion of group B donors that have the R_1R_1 phenotype compared to other ABO blood groups.

Conclusions: These results suggest that there has been an increase in the number of donors that are group B with the R_1R_1 phenotype. If exposed to the c antigen present on almost all D- red blood cell units there is an increased risk of the patient forming anti-c, which may pose an increased risk of Haemolytic Disease of the Foetus and Newborn and transfusion reactions. More data is required to understand the impact of exposure to ‘c’ when RhD negative red blood cell units are used for transfusion of RhD positive patients.

Appropriateness of transfusion of irradiated packed red cells.

Howman R[1], Howman R[1], Reemeijer P[1]
[1]*Sir Charles Gairdner Hospital, Nedlands, Australia*

Aim: At Sir Charles Gairdner Hospital, we have in place a policy that patients who are under a haematologist, on the haematology/oncology ward or attending the haematology day ward and require transfusion, will receive irradiated units due to the risk of transfusion-associated graft versus host disease in immunocompromised patients

We aimed to find the proportion of patients who received irradiated packed red blood cells (PRBC) that were not indicated.

Method: We conducted an audit of all PRBC at Sir Charles Gairdner Hospital between the months of March-April 2013. The audit was done via chart review and review of electronic databases. This project had the approval of GEKO (Governance Evidence Knowledge Outcomes) as a quality improvement project. 619 PRBC were transfused between 1 March and 30 April 2023 to 144 individual patients.

Results: According to the current ANZSBT guidelines on the Prevention of Transfusion-Associated Graft-Versus-Host Disease (May 2003), 396 of 619 irradiated red cell transfusion were indicated, with 223 irradiated transfusions not indicated. The majority of indicated transfusions were patients on nucleoside analogues (n=246), with the next largest group being patients with acute leukaemia (n=40).

Conclusion: The current policy for irradiation of blood products at our institution leads to a large number of patients receiving irradiated blood products where it is not indicated according to the current guidelines. These adds unnecessary cost, time delays and potentially greater wastage due to the shorter life span of the irradiated units. This data will inform a review of the institutional practice with regard to irradiation.

Taco Cat backwards is still Taco Cat: the importance of recognising TACO in the management of complex transfusion reactions.

Kinmonth A[1], Perchard M[1]
[1]*Royal Children's Hospital, Parkville, Australia*

Aim: Transfusion of blood products is generally safe and well tolerated, however it is not risk free. There are common risks including allergic and Febrile Non-Haemolytic Transfusion Reactions (FNHTR) through to rare and serious complications such as Transfusion Related Acute Lung Injury (TRALI). Recognition and accurate diagnosis of transfusion reactions allows for appropriate and timely management of patients, blood products and safer future transfusions.

We present a case of a complex patient and how the appropriate transfusion reaction diagnosis altered the clinical management and safety of future transfusions.

Method: We reviewed the case of a transfusion dependent seven-year-old girl with graft failure post Haemopoietic Stem Cell Transplant (HSCT). She had one transfusion reaction to platelets reported six months prior classified as a FNHTR. The treating team recommended pre-medication with antihistamine prior to platelet transfusion.

On day +73 post HSCT the treating team contacted Haematology for advice on platelet increments and mentioned three recent unreported allergic reactions to platelets. We reviewed the patient's medical record and noted the unreported reactions were respiratory focused with features of fluid overload and Transfusion Related Circulatory Overload (TACO) the more likely diagnosis.

Management changes included diuretics, slower transfusion rates and review of concurrent intravenous (IV) fluids.

Results: Our patient had six transfusion adverse events in four weeks. Three events were classified as TACO and the remaining three had respiratory features consistent with TACO and possible allergic reactions. Shifting the management focus to fluid status and rate of transfusion resulted in all subsequent transfusions being tolerated.

Conclusion:
This case demonstrates the importance of multi-team discussion and management of complex patients experiencing adverse transfusion events as the management for TACO differs from management of allergic and FNHTR. It also highlights the importance of reporting all transfusion adverse events.

BMT protocol masks indeterminate group

Mercer S[1]
[1]*NZ Blood Service, Christchurch, New Zealand*

Aim: Human error is the biggest risk to safe transfusions. Errors in patient identification during collection of the pretransfusion blood samples may lead to assigning incompatible ABO group blood components. Historical blood group results allow comparison of current test results and detection of mislabelled samples. Christchurch Blood Bank recently investigated a case where a bone marrow transplant (BMT) transfusion protocol masked a wrong blood in tube (WBiT), and New Zealand's blood management system, eTraceline, allowed the issue of red cells to the patient.

Method: Vigilant eyes scanning the eTraceline results page by chance discovered an historical WBiT whilst issuing a unit of red cells to the post BMT recipient.

BMT protocols primarily apply to ABO-mismatched allogeneic stem cell transplant recipients post engraftment. The protocol assigns a temporary ABO group of "BMT" to the patient in eTraceline, changes applicable blood group links depending on patient and donor blood groups and ignores all prior test results.

Results: In this case, both the patient and HPC donor grouped as Group A RhD negative. The WBiT sample's group was Group B RhD positive. This would normally be resulted as an indeterminate blood group given the mismatch to previous samples and BMT status. No harm came to the patient as the BMT protocol allowed the electronic crossmatch to issue Group A RhD negative blood for transfusion later that day. The circumstances of the WBiT remain under investigation.

Conclusion: eTL normally only allows group O red cells to be issued where the patient's group is indeterminate. However, the BMT status ensured that group compatible red cells were issued despite staff having previously authorised the WBiT. Review of the incident highlighted training requirements and the need to take time when issuing to check electronic crossmatching is eligible and that historical and current blood groups match.

Emergency uncrossmatched group O Blood - how is it being used at Northern Health

Murphy M[1], Hong F[1]

[1]*Northern Health, Epping, Australia*

Aim: The study was performed to analyse the appropriateness of usage of emergency uncrossmatched group O blood at a single hospital, and to describe the impact of a policy change in our blood bank to issue group O-positive blood as default for emergency circumstances in adult males and women > 50 years of age.

Method: A retrospective audit was conducted of all episodes of uncrossmatched Emergency Group O issued over a two-year period (Jan 2021 – Dec 2022) at a single hospital in Melbourne.

Results: Over the 2-year period, there were a total of 100 patients who were issued uncrossmatched Group-O blood, with 168 units issued. The change in policy to issue Group-O Rh-positive blood as default resulted in 61 units of O-negative being converted to O-positive (in a single year). Thus far, no RhD positive blood has been transfused to an RhD-negative patient. Overall, uncrossmatched blood constitutes a small (2-4%) portion of the blood bank's overall usage of Group O blood and the majority of transfusions appeared to be clinically warranted. Factors identified which lead to possibly unnecessary usage of uncrossmatched blood included – intra-operative transfusion for patients with no active crossmatch (8 episodes), rejection of crossmatch specimen (5 episodes) and transfusion for stable patients with anaemia (4 episodes), often to facilitate endoscopy. There were no recorded episodes of haemolytic transfusion reaction.

Conclusion: Emergency uncrossmatched issue is responsible for only a small amount of our laboratory's Group-O blood use. Most transfusions appeared to be clinically appropriate, although several factors were identified that could be targeted to reduce unnecessary use. The transition from default issue of O-negative to O-positive was associated with no identified complications and reduced the number of units of O-negative utilised.

Utilisation of online data capture system to improve transfusion related adverse event reporting in Western Australia.

Olsen C[1], Webster B, Yong H
[1]*Department Of Health WA, East Perth, Australia*

Aim: To improve the efficiency and accuracy of transfusion related adverse event (TRAE) reporting to Western Australian and national haemovigilance programs.

Method: An analysis of online data capture systems identified REDCap™ as a suitable platform for reporting of TRAEs by health service providers (HSPs). An online form was developed incorporating mandatory fields to ensure data captured was consistent with the Australian Haemovigilance Minimum Data Set (AHMDS). Additional fields were provided to obtain relevant information including clinical observations, symptoms, treatment, and investigations. The form was introduced in July 2021, allowing HSPs to report TRAEs in real-time. Prior to the introduction, data collection was submitted via spreadsheet at 6 monthly intervals. A review of the TRAE was completed by the HSP prior to submission. Once submitted, a final validation process was included to enable Department of Health WA (DOH WA) staff to review and if necessary, seek clarification or request additional information from HSPs before including in WA and national haemovigilance reporting programs.

Results: In the 2021-22 reporting period, 74 TRAEs were submitted via the electronic form. Using the clinical data provided, DOH WA identified 3 TRAE that required reclassification of TRAE type, the imputability rating and outcome severity were amended for 1 TRAE each, and 6 TRAEs were excluded due to not meeting the AHMDS requirements (see Figure 1). Consequently, overall data quality and compliance was improved.

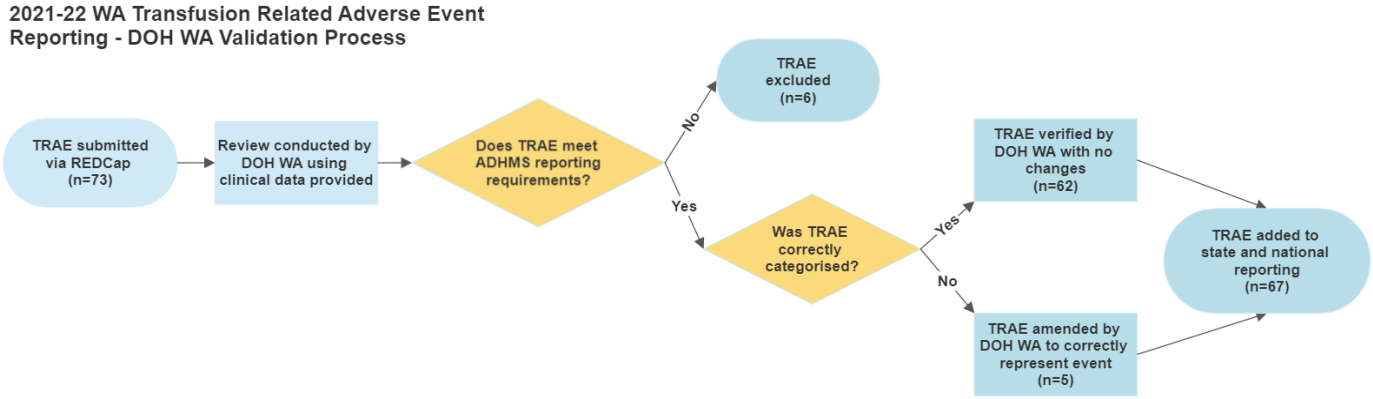


Figure 1: Validation process

Conclusion: Transition to an online data capture system has improved TRAE reporting in WA. Real-time reporting of TRAE enables HSPs to submit events when they come to light. The validation process enables DOH WA staff to review and seek clarification from HSPs, ensuring accuracy and completeness of data. As a result, monitoring emerging trends has proved simple which in turn improves patient safety.

Floods, Bushfires, and COVID-19: Impact on the Blood Supply Chain in Australia

Torres Vasconcelos F[1,2], Viennet E[2,3], Faddy H[1,2,3], Merollini K[1,4], Dean M[1,2,3], Flower R[2,3]

[1]School of Health and Behavioural Sciences, University of the Sunshine Coast, Petrie, Australia, [2]Research and Development, Australian Red Cross Lifeblood, Brisbane, Australia, [3]Faculty of Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia, [4]Sunshine Coast Health Institute, Sunshine Coast University Hospital, Birtinya, Australia

An ongoing challenge in blood supply management is maintaining adequate and safe blood stock while minimising wastage. A reliable and satisfactory supply of safe blood can be assured by a stable base of regular blood donors. However, blood supply management can be challenged by natural disasters (e.g., floods, bushfires), and pandemics (e.g., SARS-CoV-2 (COVID-19)). Natural disasters and pandemics are a concern for public health as they usually have large-scale social and economic impacts. Parts of Australia are regularly threatened by intense and unpredictable floods and bushfires, and the recent COVID-19 pandemic impacted jurisdictions throughout Australia. However, the impact of these events on the blood supply chain is not well known.

We aimed to provide an estimate of the effects of COVID-19, bushfires, and floods on the blood donor population and assess how the blood supply chain has been affected in Australia. This retrospective and descriptive regional study spans 4 years. Data has been collected on blood donations from Australian Red Cross Lifeblood (weekly donation counts from 2019 to 2022) and its management, as well as on major events. Spatio-temporal analyses will be performed on each variable (e.g., donation number, donation rate, number of deferrals, COVID-19 cases, bushfires, and floods), along with descriptive analysis, which will describe and summarise all data, and time series analysis. Time series analysis will assess the variation of count donation between phases, regions (based on SA3), week, month, year, number of COVID-19 cases, and nature of bushfire and flood.

Initial results will be presented, and issues and limitations will be discussed. Understanding the effects of these events on the blood supply will support future research on the management of the blood supply chain during disasters. It will help Lifeblood to adapt optimization strategies and sustain a sufficient and safe blood supply in the future.

Evaluation of The Clinical Use of Haemokinesis STATUS1 Group Check to Reduce the Burden on Group O Rh D Negative Blood Unit Stocks During a MTP Event

Bousounis A[1]
[1]*Eastern Health, Melbourne, Australia*

Aim: This comparison study was conducted to evaluate the clinical use of a manual system that captures a patient's group check electronically and used to reduce the burden on O Rh D Negative blood unit stock during a MTP event, by then issuing group specific units. This manual system uses the gel-card system and employs a 1-minute centrifugation for rapid forward group determination.

Method: The testing was conducted at a major hospital that has acute care services; Emergency Department, ICU, CCU and Surgical.
A total of 26 (n=26) first presentation patient samples were tested as simulated emergency patients upon activation of a Mass Transfusion Protocol (MTP).
The concordance of the results and TAT was observed and recorded.

Results: The comparison between timings or turn-around-time (TAT) was conducted between two systems. One outlier identified of 13 minutes which was still included in the data to help simulate real laboratory conditions where staff constantly multi-task.

Conclusion: Using a system that can provide a rapid (4 minute), accurate and electronically recorded whole blood forward group result, could assist patients that require urgent blood during for MTP; they can be issued with group specific blood units as opposed to scarce O Rh D Negative blood units. Further to this, implementing a system with a high TAT, can be utilised to reduce the risk of sensitisation and production of alloantibodies to the D antigen if O Positive blood units are alternatively issued for MTP.

An examination of fresh frozen plasma wastage: an audit of a tertiary care hospital in NSW

Brenu E[1,2], Shuttleworth C[2,3,4]
[1]St George Hospital, Kogarah, Australia, [2]St George and Sutherland Clinical School, UNSW, Sydney, Australia, [3]St George Hospital, Department of Haematology, Kogarah, Australia, [4]The Sutherland Hospital, Department of Haematology, Caringbah, Australia

Aim: Despite the existence of stringent guidelines defining the indications for fresh frozen plasma (FFP) in tertiary centres, FFP wastage is a common occurrence. The purpose of this audit was to explore the causes of inappropriate wastage of FFP and the clinical departments where this occurs in a tertiary hospital.

Method: This was a retrospective study that examined FFP orders that were wasted between 2017 and 2022 at Sutherland Hospital in Australia. The following information were collected on all orders; features of the patient for whom the FFP orders were made (including patient demographics, clinical history, and number of FFP requested), indications for FFP, departments requesting FFP, and the reason for wastage. Eighty-eight patients were included in this audit. Reasons for FFP wastage were classified into death, discontinuation of products, incomplete administration of products, failure to administer products, and patient transfer between departments/institutions. Data collection and quantitative analysis was performed using Microsoft excel 2022 and simple statistics in SPSS 22.0 respectively.

Results: A total of 224 FFPs were requested for 88 patients between 2017 and 2022 that were not used. Wastage of FFP mainly occurred during transfer of patients (43%) between departments or institutions and this occurred predominantly when patients were transferred from the emergency department (39%). Other departments with high level of wastage include critical care unit (22%) and surgical departments (20%). FFP wastage was less common in the operation theatre (10%), medical (8%), obstetrics and gynaecology departments (1%).

Conclusion: Substantial wastage of FFP is common in many institutions and this audit demonstrates that wastage largely occurs due to lack of communication between departments particularly during transfer of patients from the emergency care setting to other departments or institutions.

Changes in preoperative anaemia and perioperative red cell transfusion practice for patients undergoing major surgery- a 13-year review

Sinha R[1], **Gore C[1]**, Ireland S[1]
[1]Blood, Organ & Tissue Programs, SA Health, Adelaide, Australia

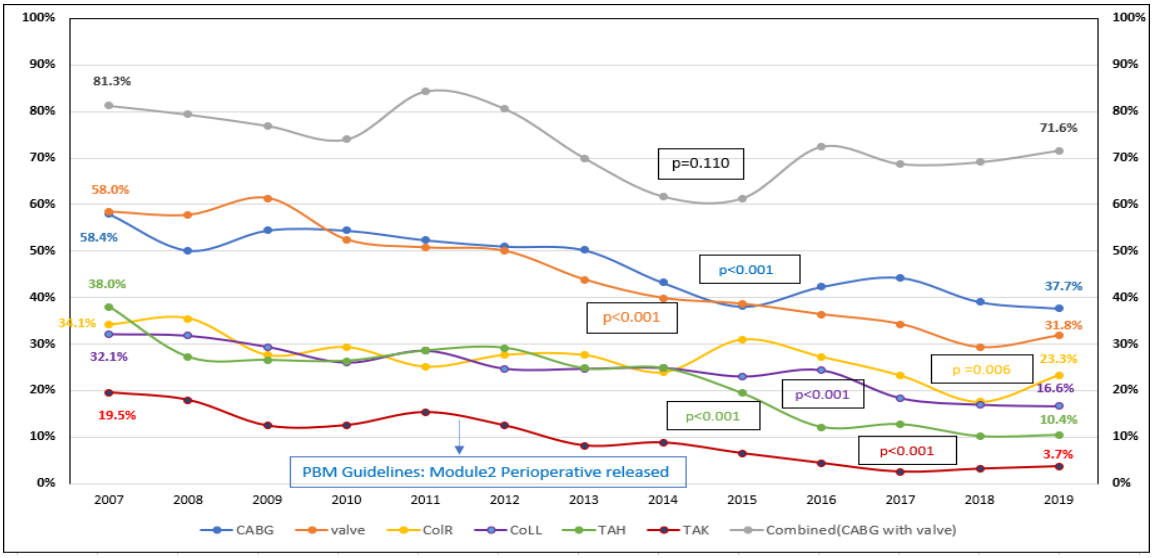
Aim: Pre-operative anaemia is a strong predictor for perioperative blood transfusion requirements and administration of blood in the perioperative setting is a risk factor contributing to poor outcomes. A retrospective study was undertaken to review the changes in the incidence of pre-operative anaemia and red cell transfusion in patients undergoing cardiothoracic, colorectal, and orthopaedic surgery admissions at major hospitals over a 12-year period.

Methods: Data was collected from an electronic database that links South Australian (SA) public hospitals data with selected laboratory test data, including blood and blood product data. Admissions for major surgical procedures at five metropolitan hospitals from 2007-08 to 2019-2020 financial years were included. Red cell transfusions during admission and haemoglobin levels eight weeks before surgery and the closest to the hospital admission were analysed.

Results: A total of 34,685 surgical admissions were analysed. Procedures included arthroplasty of hip [THR] (7104), arthroplasty of knee [TKR] (8238), right sided colorectal procedures (ColR, 2780), left sided colorectal procedures (ColR, 4437), coronary artery bypass [CABG] (7056), valve surgery (3734) and combined CABG with valve surgery [Combined,1336] were identified. The overall pre-operative anaemia rates decreased from 33.1% in 2007-08 to 21.7% in 2019-20 ($p<0.001$), with a significant decrease from 36.3% to 24.8% in CABG, 36.1% to 24.7% in ColL, 65.7% to 53.6% in ColR, 20.3% to 12.9% in THR and 17% to 10% in TKR. The overall transfusion rate decreased from 41.6% in 2007-08 to 20.2% in 2019-20 ($p<0.001$), including a significant decrease found in all the procedures except the Combined procedure (Fig 1).

Conclusion: The findings suggest that pre-operative anaemia and transfusion rates have decreased significantly in the last decade. This change may reflect improved adoption of patient blood management, including pre-operative anaemia management, haemostasis monitoring, conservation initiatives (e.g, use of cell salvage, improved surgical techniques), and restrictive transfusion.

Fig 1: Transfusion rates for all selected procedures over the 13-year period


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
81.3%
71.6%
p=0.110
58.0%
58.4%
p<0.001
38.0%
37.7%
34.1%
p<0.001
31.8%
32.1%
p =0.006
23.3%
p<0.001
16.6%
19.5%
p<0.001
10.4%
p<0.001
3.7%
PBM Guidelines: Module2 Perioperative released
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
CABG
valve
ColR
CoLL
TAH
TAK
Combined(CABG with valve)

Cryoprecipitate usage in ROTEM Delta vs ROTEM Sigma guided transfusions in a major haemorrhage protocol.

Ling J[1], Gangatharan S[1], Moraes C[1], Kaminskis L[1], P'Ng S[1]
[1]*Fiona Stanley Hospital, Perth, Australia*

Aim: Incorporation of thromboelastometry point-of-care testing into major haemorrhage protocol (MHP) may be beneficial in patients with critical bleeding. The ROTEM Sigma coagulation analyser is a fully automated point-of-care device replacing the ROTEM Delta. Variation was found between FIBTEM assays of ROTEM Sigma and ROTEM Delta.[1] We aim to determine if ROTEM Sigma increases rates of cryoprecipitate transfusions compared to ROTEM Delta in major haemorrhage.

Method: Our institutions MHP recommends transfusing cryoprecipitate where Clauss fibrinogen is <1.5g/L or ROTEM FIBTEM A5 < 10mm. ROTEM Delta analysers changed to ROTEM Sigma on 2nd of December 2021. We retrospectively collected data on patients aged 16+ who had ROTEM Delta (between January 2021 to 2nd December 2021) or ROTEM Sigma (between 1st January 2022 to 31st October 2022) performed during MHP activation.

Results: Of 307 total MHP activations, 68% of patients had either ROTEM Delta (n=103) or ROTEM Sigma (n=105) performed.

In the ROTEM Delta cohort, mean FIBTEM A5 was significantly higher at 16.4mm (range 3-51) vs 12.6mm (range 2-31) in ROTEM® Sigma (p=0.0012). FIBTEM A5 was <10mm in 25% of patients with ROTEM Delta compared to 34% with ROTEM Sigma (p=0.1681).

Mean cryoprecipitate transfused for ROTEM Delta was 0.60 vs 0.82 adult doses with ROTEM Sigma (p=0.2495). 80% of patients with FIBTEM A5<10mm had cryoprecipitate transfusions in ROTEM Delta compared to 60% in ROTEM Sigma (p=0.159).

Where there were paired fibrinogen and ROTEM samples, correlation coefficient for the ROTEM Delta (r=0.89; n=54) and ROTEM Sigma (r=0.88; n=59) were similar.

Conclusion: Mean FIBTEM A5 was lower with ROTEM Sigma, however this did not result more patients with FIBTEM A5<10mm, or increased cryoprecipitate use. Changing ROTEM triggers for transfusion in our MHP is not required with ROTEM Sigma analysers.

References:

- Gillissen A, et al. Comparison of thromboelastometry by ROTEM Delta and ROTEM Sigma in women with postpartum haemorrhage. *Scandinavian J of Clinical and Laboratory Investigation*. 2019; 79:32-38.

Comparing the Efficiency of Smart Blood Fridge Systems and Traditional Practices in Transfusion Metrics

Mann R[1], Whiting P[1], Di Ciaccio P[1,2,3]
[1]Capital Pathology (Sonic Healthcare Australia), Canberra, Australia, [2]Australian National University, Canberra, Australia, [3]The Canberra Hospital, Canberra, Australia

Aim: To compare the efficiency of automated, semiautomated, and manual blood fridge release systems in a network of peripheral hospitals.

Method: Unique in Australia, Capital Pathology (a division of Sonic Healthcare) serves a network of private hospitals in the Australian Capital Territory (ACT) utilising three varying blood fridge systems: a semi-automated standalone BloodTrack kiosk (Haemonetics Corporation, MA, USA), a manual blood fridge, and a complete Haemobank 20 remote release BloodTrack system (Haemonetics Corporation, MA, USA/Helmer Scientific, IN, USA). The Haemobank 20 system was first installed in 2017, and the standalone BloodTrack Kiosk in 2019. We analysed transfusion records from 2020 to 2022 from these hospitals serviced by Capital Pathology. We focused on the annual crossmatch to transfusion ratio (C/T ratio) and the rate of single-unit transfusions. This data was examined to deduce the efficiency of each blood fridge system in the laboratory setting.

Results: Over the three-year period, the Haemobank 20 system demonstrated the lowest overall C/T ratio and the highest rate of single-unit transfusions. Conversely, the semi-automated BloodTrack kiosk showed the highest C/T ratio and the lowest rate of single-unit transfusions.

Conclusion: The fully automated Haemobank 20 system exhibited superior performance in C/T ratios and single-unit transfusions, suggesting potential benefits for workflow optimisation, reduced red cell wastage, and decreased laboratory travel times and costs. Haemobank 20 is also completely automated, reducing the likelihood of clerical error. Despite its hybrid design, the BloodTrack kiosk system showed the least optimal performance in the metrics analysed. Haemobank 20 is an efficient blood fridge system, particularly for hospitals with offsite blood bank laboratories.

Supporting the clinical need for Gerbich negative blood in Papua New Guinea

Jacko G[1], **Moreno A[1]**, Powley T[1], Davison C[1], Mathias M[2], Ivarato P[2], Liew Y[1], Wilson B[1], Roots N[1], Millard G[1], Martin J[1], Daly J[1,3]
[1]Australian Red Cross Lifeblood, , Australia, [2]National Blood Service , Port Moresby, Papua New Guinea, [3]Blood transfusion Genomics Consortium, ,

Aim: High prevalence Gerbich antigens are expressed on glycophorins C and D of red blood cells. The highest prevalence of Gerbich negative phenotypes have been reported in Papua New Guinea (PNG).[1] This is suspected to be as a result of Gerbich negative erythrocytes providing partial protection against malaria, which is endemic to PNG.[2] Gerbich antibodies can cause haemolytic disease of the fetus and newborn (HDFN) and haemolytic transfusion reactions (HTR).[2] Yet, identifying Gerbich negative blood donors can be difficult and costly due to the high prevalence and limited reagents for serologic testing. This study aims to identify Gerbich negative blood donors in PNG, using serologic and molecular methods, to aid transfusion services in providing Gerbich negative blood products when needed.

Method: Blood samples collected from randomly selected consenting blood donors who successfully donated at the Port Moresby General Hospital Blood Transfusion Service between January and May 2023 were transported to Australian Red Cross Lifeblood for testing. Gerbich negative phenotypes were determined by serologic testing using in-house antisera. Additionally, molecular testing, using the Universal Blood Donor GenoTyping (UBDT) research microarray[3], and genotype calling, using the AxiomGT1 algorithm[4], were used for comprehensive blood group results, including analysis of the copy number region plots of *GYPC* gene to determine the predicted Gerbich phenotype. Negative, atypical, and discordant Gerbich results were also investigated using short-read sequencing.

Results: Preliminary serologic testing has identified 11 Gerbich negative donors from the first 850 samples received. Results of broader red cell genotyping and *GYPC* sequencing will be presented.

Conclusion: Identification of Gerbich negative blood donors in PNG will assist the National Blood Service to provide suitable blood products when required. Moreover, further analysis of the comprehensive blood group results for these PNG blood donors may assist broader blood product provision and identify further transfusion service priorities.

References

1. Booth PH, McLoughlin K. The Gerbich Blood Group System, Especially in Melanesians. *Vox Sanguinis* 1972;22:73-84.
2. Jaskiewicz E, Peyrard T, Kaczmarek R, Zerka A, Jodlowska M, Czerwinski M. The Gerbich blood group system: old knowledge, new importance. *Transfus Med Rev*. Apr 2018;32(2):111-116. doi:10.1016/j.tmrv.2018.02.004
3. Gleadall NS, Veldhuisen B, Gollub J, et al. Development and validation of a universal blood donor genotyping platform: a multinational prospective study. *Blood Adv*. Aug 11 2020;4(15):3495-3506. doi:10.1182/bloodadvances.2020001894
4. Axiom Analysis Suite v5.1. User Guide. Santa Clara, CA: Thermo Fisher Scientific; 2020.

Knops blood group system single nucleotide variants (SNVs) in an African population – implications for red cell investigations

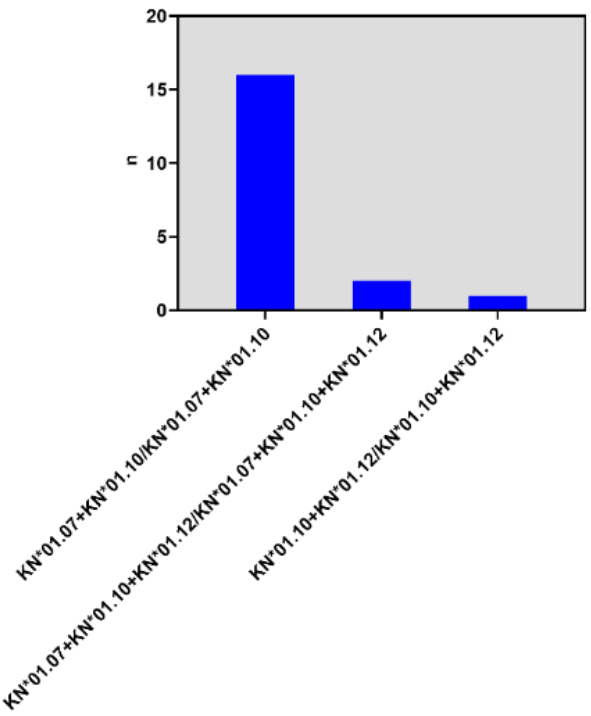
Perros A[1], Githiomi R[2,3], Roulis E[1,4], Sowah S[1], Flower R[1,4], Hyland C[1]
[1]Research & Development, Australian Red Cross Lifeblood, Brisbane, Australia, [2]Department of Health, Policy and Research, Ministry of Health, , Kenya, [3]Department of Medical Laboratory Sciences, Medical School, Mount Kenya University, , Kenya, [4]Faculty of Health, Queensland University of Technology, Brisbane, Australia

Aim: Antigens of the Knops blood group system are located on complement receptor 1 (*CR1*) and can be difficult to identify due to variability in red cell reactivity. Knops antibodies can mask the presence of, and mimic clinically significant antibodies. Genomic technology can alleviate these difficulties by sequencing single nucleotide variants (SNVs) associated with Knops antigens. We reviewed Knops associated SNVs in a Kenyan population.

Method: Genomic DNA from 191 blood donors in Kenya were genotyped by next generation sequencing performed at Lifeblood using a targeted custom blood group sequencing panel on the Illumina MiSeq[1]. Knops genotype determined using variant call format files mapped to reference genome hg19. ISBT Blood Group Allele Tables were used to determine predicted phenotype.

Results: Two samples were heterozygous for a stop-codon upstream of the Knops antigenic region encoded by c.3106C>T (rs771592989). This is a rare variant in all populations. Variants encoding Knops alleles in our cohort had similar frequencies to those reported for the African population in ExAC and gnomAD. However, the frequency of Knops alleles *KN*01.-05* and *KN*01.12* in our cohort were comparatively lower. An unusual combination of multiple variants encoding Knops alleles in study participants was also observed (Figure 1).

Figure 1. Unusual Knops antigen combinations



Conclusion: Significant genetic variability in *CR1* was evident in our cohort. Multiple and unusual combinations of Knops alleles, and an upstream stop-codon were observed. These variants may play a role in malarial infection, as *CR1* and Knops antigens are associated with the severity of *Plasmodium falciparum* infection[2, 3]. The genetic variability of the Knops blood group system evident in our cohort may lead to complications in serological red cell typing where patients present with multiple red cell antibodies. The use of sequencing technologies is key for solving the complexities associated with the Knops system and could be used to further elucidate the association with malarial infection.

References

1. Roulis, E., et al., *Targeted exome sequencing designed for blood group, platelet, and neutrophil antigen investigations: Proof-of-principle study for a customized single-test system.* Transfusion, 2020. 60(9): p. 2108-2120.
2. Daniels, G., *Knops Blood Group System and the Cost Antigens*, in *Human Blood Groups*. 2013. p. 439-448.
3. Hansson, H.H., et al., *Human genetic polymorphisms in the Knops blood group are not associated with a protective advantage against Plasmodium falciparum malaria in Southern Ghana.* Malaria Journal, 2013. 12(1): p. 400.

Results of an audit on use of Cytomegalovirus (CMV) negative blood in the Hunter New England Health district

Ringkowski S[1,2,4], McGarity A[3], Liang H[3], Rankin K[1,2], Partridge B[1], Dimmock S[1], Ross B[1,5], Enjeti A[1,2,4]

[1]NSW Health Pathology, New Lambton Heights, Australia, [2]Calvary Mater Newcastle Haematology, Waratah, Australia , [3]Hunter New England Health District, New Lambton Heights, Australia, [4]University of Newcastle, Callaghan, Australia, [5]The Children's Cancer and Haematology Service, The John Hunter Children's Hospital, New Lambton Heights, Australia

Aim: To evaluate the adherence of prescription of CMV negative blood to local and national guidelines.

Method: All episodes of provision of requested CMV-negative blood to patients provided between 15/01/2018 and 20/06/2022 (excluding neonatal, intrauterine and transfusions in pregnancy) were retrieved. Clinical notes were reviewed to assess if transfusion was "indicated per local policy" (CMV-negative adult bone marrow transplant patients, paediatric patients requiring immunosuppressive therapy (IT), stem cell therapies (SCT) or severely immunosuppressed (SI) patients) or "can be considered" or "not indicated" per national guidelines. CMV serology status of the recipient was recorded where available**.** The "can be considered" group included "solid organ transplant" and other "haematology/oncology patients".

Results: A total of 499 CMV-negative blood products were released from John Hunter blood bank to 293 different patients during this timeframe. Of the 293 patients receiving transfusions, 34.1% were "indicated per local policy" and comprised of 18 CMV-negative adult bone marrow transplant patients as well as 82 paediatric IT, SCT and/or SI patients. 61.8% of patients were in the "can be considered" group. Of these, 126 were solid organ transplant recipients and 85 (67.5%) were CMV-positive. 48 patients were haematology/oncology but not SCT patients and of these 21 (43%) were CMV positive. Seven patients were adult CMV-positive stem cell transplant recipients. 4.1% were classified as not indicated.

Conclusion: The majority of CMV-negative blood transfusions were not indicated as per local or national guidelines. A review of local policy with education about indications for use of CMV-negative blood are some of the strategies that may help with optimal usage.

Preserving patients own blood through implementation of low volume blood tubes in an acute in-patient oncology/haematology unit

Simonsen C[1], Barbur B[1], Rothery M[1,3], Im S[1,2], Kovacs I[1], Nugent K[1], Wenn B[1]
[1]Princess Alexandra Hospital, Brisbane, Australia, [2]University of Tasmania, Hobart, Australia, [3]Ipswich Hospital, Ipswich, 4305

Aims: Oncology/haematology patients experience a high frequency and volume of blood collections in the acute hospital setting, contributing to iatrogenic anaemia. These patients have impaired haematopoiesis due to direct effects of anti-cancer treatments and disease processes, making them vulnerable to additional blood losses. A quality improvement initiative was undertaken to preserve patients own blood.

Methods: The intervention was developed through staff engagement strategies and a literature review which identified low volume blood tubes as an effective and acceptable intervention to minimise blood loss.

Blood sample data from the inpatient unit was analysed pre intervention to identify high frequency and volume blood tubes. Low volume equivalent tubes were implemented for all patients (n=294) over a 19-month trial period. Blood sample data was analysed after the trial period to estimate and compare total blood volume collected using standard blood tubes and low volume blood tubes. Acceptability of the innovation was monitored via staff feedback forms, pathology turnaround times and no test results.

Results: Estimated total blood volume using standard blood tubes was 43.8L (149mL per patient) compared to 23.2L (78mL per patient) using low volume tubes. This demonstrates an estimated 20.6L (52.8%) reduction in total blood volume; 71mL preserved blood per patient.

Limitations include assumptions that blood volume collected was equal to maximum blood tube volume and variations in collection techniques may exist. Iatrogenic anaemia is multifactorial and therefore it is not possible to directly correlate low volume tubes with reduced iatrogenic anaemia however these trial results suggest the aim was achieved.

Conclusion: Low-volume blood tubes present an acceptable alternative to standard blood tubes in the in-patient cancer setting. There is future scope to preserve patients own blood through implementing this practice change in outpatient cancer settings, as well as additional strategies such as reduced frequency of blood sampling in subsequent stages of this project.

Plasma Exchange followed by IVIg to manage Haemolytic Disease of the Fetus and Newborn: 2 cases

Wise T[2], Gheysen W[3], Polidano G[2], Hogan C[2], Fung A[3], Skrzypek H[3], Willcox A[1,2]

[1]ACBD, Monash University, Melbourne, Australia, [2]Austin Hospital, Heidelberg, Melbourne, Australia, [3]Mercy Hospital For Women, Heidelberg, Melbourne, Australia

Background: Haemolytic Disease of the Fetus and Newborn (HDFN) occurs when maternal blood group antibodies cross the placenta during pregnancy destroying the fetal red cells[1,2]. Severe fetal anaemia can result in hydrops and in-utero demise. Whilst intra-uterine transfusion (IUT) can be performed, the optimal strategies for managing severe HDFN are not well defined[2]. Here we present two cases where sequential plasma exchange (PLEX) followed by IVIg was used to manage HDFN.

Case Description:

Case 1: 33-year-old, G6P3 with blood group B RhD+, K- with a high titre anti-K antibodies (1:1024 at 37℃ IAT) was referred to haematology at 13/40 having received IUTs with her prior two pregnancies. Non-invasive prenatal analysis (NIPA) confirmed that the fetus was K+. At 14/40 she commenced PLEX alternate daily for 7 days followed by IVIG 1g/kg weekly until 28/40 which effectively reduced the antibody titre. Fetal anaemia did not occur and no IUT was required. A healthy baby boy was delivered at 37/40 (Hb 123g/L).

Case 2: 37-year-old, G6P4(-1), referred to haematology at 16/40, was group A RhD- (C-/c+/S-/s+) with high titre anti-D antibodies (1:4096 at 37℃ IAT) along with anti-C and anti-S antibodies (1:4 at 37℃ IAT). She previously required IUTs. Her 5th pregnancy resulted in hydrops-related neonatal death at 24/40 without timely obstetric care. NIPA confirmed the fetus as A, RhD+, C+ for her current pregnancy. She received 3 PLEX followed by IVIG 1g/kg weekly which reduced the anti-D titre to 1:1024. At 26/40, the anti-D titre doubled (1:2048) and the fetus was anaemic requiring an IUT.

Discussion: Optimal approach to reduce the risk of HDFN is not known[1], here we describe two cases where immunomodulation with PLEX and IVIg reduced maternal antibody titres and abrogated the need for IUT in one case and maintained a pregnancy to safe IUT date in another.

1. Delaney, M. & Matthews, D. C. Hemolytic disease of the fetus and newborn: managing the mother, fetus, and newborn. *Hematology* 2015, 146–151 (2015).
2. Winter, D. P. de, Kaminski, A., Tjoa, M. L. & Oepkes, D. Hemolytic disease of the fetus and newborn: systematic literature review of the antenatal landscape. *Bmc Pregnancy Childb* 23, 12 (2023).

256 (-headings and references)

Developing a computerised clinical decision support tool for massive transfusion

Sanderson B[1], Field J[1], Kocaballi A[2], Estcourt L[3], Magrabi F[4], Wood E[5], Coiera E[4]
[1]Department of Anaesthesia and Perioperative Medicine, Westmead Hospital, Westmead, Australia, [2]School of Computer Science, University of Technology, , Australia, [3]NHS Blood and Transplant, , UK, [4]Centre for Health Informatics, Australian Institute of Health Innovation, , Australia, [5]School of Public Health and Preventive Medicine, Monash University, , Australia

Aim: Management of major haemorrhage with massive transfusion (MT) requires significant hospital resources and mobilisation of a multidisciplinary team that is placed under significant cognitive load. Clinical decision support (CDS) tools have been used successfully to reduce cognitive load and improve patient outcomes but the role of CDS in MT is not well established. We aimed to develop a CDS for MT and evaluate its impact on the efficacy and efficiency of decisions made during a simulated MT.

Method: Multicentre, multidisciplinary, user-centred design study to develop a computerised CDS for MT, *MTP Assistant*. This was compared to a paper-based MT protocol for management of major haemorrhage in a subsequent randomised simulation trial.

Results: 18 staff relevant to MT were recruited and assisted in designing a computerised CDS for MT with integrated aetiology-specific treatment protocol recommendations, live interpreted laboratory results and blood product tracking. 44 critical care doctors and nurses were subsequently randomly allocated to teams to complete two 20-minute major haemorrhage scenarios using both *MTP Assistant* and a paper-based protocol. Compared to paper-based management, *MTP Assistant* demonstrated improved decision velocity (mean 8.5 decisions per hour, versus 6.9, p 0.003) for MT-related decisions with similar efficacy (mean 13.3 versus 13.2 correct decisions, p 0.92) whilst reducing cognitive load and with excellent usability.

Conclusion: A CDS for MT must support multiple physically separated staff to maintain current scenario information and allow concurrent user interaction to address these highly complex clinical scenarios. When compared to paper-based management, CDS for MT was able to improve performance of clinical decision processes whilst reducing cognitive load.

A Multidisciplinary Model for Prioritizing Patient Safety with Paediatric Anticoagulation Stewardship

Barnes C[1], Greenway A[1], Krake E[1], Gilmore H[1], In M[1]
[1]***Royal Children's Hospital, Parkville, Australia***

Aim: Anticoagulation therapy in paediatric patients poses unique challenges due to the increasing number and complexity of cases. Recognizing this need, the Royal Children's Hospital has implemented the first sustainable and multidisciplinary anticoagulation stewardship program in a paediatric hospital in Australia. Aligned with recommendations from the Australian Commission on Safety and Quality in Healthcare and the Joint Commission on Accreditation of Healthcare Organizations, this paper presents the initial activities, findings and outcome of the first 12 months of the anticoagulation stewardship program.

Method: The three goals of the anticoagulation stewardship were developed to include:

- Ensure patient safety.
1. Optimise anticoagulation therapy.
2. Enhance collaboration among departments.

These goals were to be achieved within the following framework:

Results: Anticoagulation stewardship engages in regular stewardship meetings to identify patient safety events and has established a Clinical Anticoagulation Working Group (CAWG) to address complex anticoagulation issues. Furthermore, the program emphasizes interdepartmental collaboration by fostering partnerships with key stakeholders. Through needs analysis meetings, the anticoagulation stewardship team has engaged with departmental heads to ensure a comprehensive approach to patient care.

Conclusion: The implementation of the paediatric anticoagulation stewardship program at the Royal Children's Hospital demonstrates a proactive and effective model for patient safety. By integrating a multidisciplinary team, fostering collaboration, and establishing clear protocols, this program serves as a valuable framework for improving anticoagulation safety in paediatric healthcare settings.

Determining the relationship between platelet size and glycoprotein expression by flow cytometry to assist phenotypic characterisation of patients with suspected inherited platelet disorders.

Connor D[1], Morel-Kopp M[2,6], Campbell H[5], Donikian D[3,4], Kondo M[3,4], Chen W[2,6], Liang H[5], Stevenson W[2,6], Chen V[5], Joseph J[1], Brighton T[3,4], Ward C[2,6], Rabbolini D[2]
[1]St Vincent's Hospital, Sydney, Australia, [2]Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, Australia, [3]Prince of Wales Hospital, Sydney, Australia, [4]Haematology NSW Health Pathology Randwick, Sydney, Australia, [5]ANZAC Research Institute and Concord Repatriation Hospital, Concord, Australia, [6]Northern Blood Research Centre, Kolling Institute of Medical Research, Sydney, Australia

Aim: In patients with suspected inherited platelet disorders (IPDs), precise phenotypic data is needed to assign pathogenicity or prioritise referral for further functional or genetic analysis. We sought to determine correlations between changes in platelet-glycoprotein (pGP) expression and platelet size to characterise the significance of changes in pGP expression.

Method: Flow cytometry was used to measure pGP expression for 130 individuals recruited to the Sydney Platelet Group between 2017 and 2022. Platelet size estimation by forward scatter (FSC) was performed in 120 cases. The analysis cohort was divided into groups based on platelet number, size and abnormalities expected to change pGP expression. Differences in platelet size between groups was determined by Kruskall-Wallace testing followed by Dunn's post-hoc multiple pairwise comparisons. The correlation between platelet FSC and pGPs was computed using Spearman's correlation coefficient, and linear models were constructed to determine the significance of correlation. Regression plots for pGP and FSC were constructed to interpret deviations from expected pGP expression based on cell size.

Results: Platelet size distribution was significantly different between patients with macrothrombocytopenia and controls, and between patients with normal sized platelets. Significant correlations were found between pGPs, CD36(GPIV), CD31(PECAM1), CD110(MPL), CD9, CD41(GPIIb), CD61(GPIIIa), CD42a(GPIX), CD42b(GPIb□) and FSC in controls. Linear modelling confirmed significant correlation between pGP expression and FSC to enable estimates of pGP change per 1 unit increase in FSC (Table 1). Subsequent regression curves supported the significance of reduced pGP expression caused by 2 novel variants in GP1BA in 4 individuals and 1 variant in ITGA2B in 1 individual.

Glycoprotein expression	CD41(GPIIb)	CD61(GPIIIa)	CD42a(GPIX)	CD42b(GPIb□)
Patients with macrothrombocytopenia	0.25	0.32	0.33	0.31
Controls	0.25	0.08	0.31	0.23

Conclusion: Specific pGP expression is correlated to changes in platelet FSC. Models can be established to prioritise patients with suspected abnormalities for further phenotypic or genetic analysis.

Fibrinolysis shutdown and kinetics of normalisation in a critically unwell patient: a case report from the VETtiPAT clinical trialists.

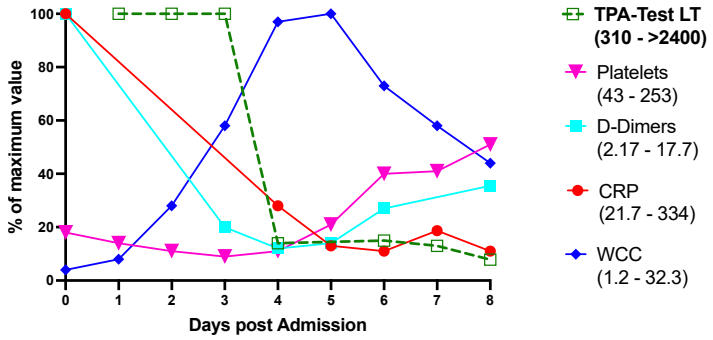
Coupland L[1,2,3], Rabbolini D[4,7], Crispin P[5,6], Aneman A[1,2,3]
[1]Ingham Institute Of Applied Medical Research, Liverpool, Australia, [2]Intensive Care Unit, Liverpool Hospital, Liverpool, Australia, [3]South West Sydney Medical School, UNSW Medicine, Liverpool, Australia, [4]Kolling Institute of Medical Research, The University of Sydney, St Leonards, Australia, [5]Haematology Dept, The Canberra Hospital, Garran, 2605, [6]The John Curtin School of Medical Research, The Australian National University, Acton, Australia, [7]Oxford Haemophilia and Thrombosis Centre, Headington, United Kingdom

Aim: Abnormal fibrinolysis in critically ill patients may lead to widespread thrombosis and multi-organ failure. We are exploring the use of ClotPro® viscoelastic testing (VET) to identify fibrinolysis resistance and shutdown. We aim to establish the pathophysiology, identify clinical laboratory correlates and explore the efficacy of tissue plasminogen activator (tPA) and/or plasminogen in normalising the defect.

Method: A case study that illustrates the utility of ClotPro TPA-test to detect fibrinolytic shutdown. VET trends were correlated to routine laboratory and clinical parameters. In-vitro spiking of the TPA-test with tPA (650–1300 ng tPA/mL blood) and/or lyophilised plasminogen (147 µg/mL) was performed.

Results: A 36 yo male presented in extremis with Strep. pyogenes pneumonia complicated by acute respiratory failure, multi-organ dysfunction, leukopenia and possible DIC (thrombocytopenia ($55x10^9$/l), raised D-dimers (18) and prolonged PT (21secs)). ClotPro TPA-test confirmed fibrinolysis shutdown (lysis time >2400sec). In-vitro supplementation of blood with plasminogen, but not tPA, successfully induced lysis. This effect was maintained for 24hrs. D-dimer's reduced dramatically between admission and Day 3, coinciding with the phase of fibrinolysis shutdown. Fibrinolysis normalisation was seen on Day 4 of admission preceding modest increases in D-dimer levels, and improvements in clinical condition and platelet count.

Conclusion: Fibrinolysis status is not static in critical illness and changes can be detected and monitored by the ClotPro TPA-test. In this setting, low D-dimer levels may indicate fibrinolysis shutdown and, therefore, not reflect thrombotic risk. The ClotPro TPA-test may offer a highly sensitive marker of the trajectory of diseases driven by inflammation, tissue damage and hypoxia. Vital and cutting-edge ongoing research will explore changes in fibrinolysis in critically ill patients, the association with DIC scores, response to tPA or plasminogen in-vitro and fibrinolysis protein analysis.



Final data from the PREVENT study evaluating real-world usage and effectiveness of a recombinant factor VIII Fc and recombinant factor IX Fc in haemophilia A or B in Germany

Bidlingmaier C[1], **Curnow J[2]**, Heller C[3], Langer F[4], Miesbach W[5], Scholz U[6], Oldenburg J[7], Röhss J[8], Falk A[8], Santagostino E[9], Tiede A[10]
[1]Dr. von Hauner's Children's Hospital, Paediatric Haemophilia Centre, Munich, Germany, [2]Westmead Hospital, Sydney, Australia, [3]University Hospital of Frankfurt, Department of Pediatric Hematology/Oncology, Frankfurt, Germany, [4]University Medical Centre Hamburg-Eppendorf, II. Medical Clinic and Polyclinic, Hamburg, Germany, [5]Goethe University Hospital, Medical Clinic II, Institute of Transfusion Medicine, Frankfurt, Germany, [6]Center of Coagulation Disorders, Leipzig, Germany, [7]University Clinic Bonn AöR, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany, [8]Sobi, Stockholm, Sweden, [9]Sobi, Basel, Switzerland, [10]Hannover Medical School, Department of Hematology, Haemostasis, Oncology and Stem Cell Transplantation, Hannover, Germany

Aim: The safety and efficacy of efmoroctocog alfa (herein rFVIIIFc) and recombinant factor IX Fc fusion protein (rFIXFc) for the treatment of haemophilia A and B, respectively, have been demonstrated in pivotal Phase 3 and extension studies. The non-interventional PREVENT (NCT03055611) study aimed at describing the real-world usage and effectiveness of prophylactic treatment with rFVIIIFc and rFIXFc in Germany over a 24-month prospective period.

Methods: Enrolled patients were already on rFVIIIFc/rFIXFc treatment or were prescribed rFVIIIFc/rFIXFc at study entry. At enrolment, patient characteristics and retrospective data were collected. The primary endpoints were annualised bleed rate (ABR), prescribed injection frequency (IF), and dispensed factor consumption. Only patients with ≥3 months treatment duration were included in the analyses of annualised endpoints. Data were analysed using descriptive statistics.

Results: The analysis included 150 people with haemophilia A (PwHA; 1 female) and 47 people with haemophilia B (PwHB) (Table 1).

During the prospective follow-up, median (IQR) ABR was 0.5 (0.0-1.7) on rFVIIIFc prophylaxis (n=149) and 1.8 (0.0-4.6) on rFIXFc prophylaxis (n=47). Mean (SD) weekly IF was 2.5 (0.7) and 1.2 (0.4) injections per week, respectively, with a mean (SD) weekly factor consumption of 91.9 (42.3) IU/kg/week rFVIIIFc (n=147) and 56.2 (34.7) IU/kg/week rFIXFc (n=45).

The average proportion of patients experiencing 0 bleeds during 6-month intervals of prospective follow-up increased from 65.3% to 79.2% for rFVIIIFc and from 45.7% to 60.0% for rFIXFc-treated patients.

rFVIIIFc and rFIXFc were well tolerated with no serious adverse events related to treatment reported during the prospective period.

Conclusion: The PREVENT study showed that prophylactic treatment with rFVIIIFc/rFIXFc provided good protection from bleeds with low injection frequencies and factor consumption in the expected range over 20.6/21.0 months of prospective follow-up.

Table 1: Patient demographics and baseline characteristics at enrolment

	Haemophilia A (n=150)	Haemophilia B (n=47)
Age (years), median (range)	21.0 (0–74)	26.0 (2–78)
Age category (years), n (%)		
<12	44 (29.3%)	9 (19.1%)
12–18	21 (14.0%)	6 (12.8%)
19–64	83 (55.3%)	29 (61.7%)
≥65	2 (1.3%)	3 (6.4%)
Severity of haemophilia, n (%)		
Severe	132 (88.0%)	42 (89.4%)
Moderate	17 (11.3%)	4 (8.5%)
Mild	1 (0.7%)	1 (2.1%)
Treatment status at enrolment		
Prior rFVIIIFc/rFIXFc, n (%)[a]	128 (85.3%)	35 (74.5%)
Prospective follow-up (months) prophylaxis, median (range)	20.6 (0.5–30.0)	21.0 (3.9–29.7)

Percentage values may not sum to 100 due to rounding. [a]All patients were tolerised prior to enrolment.

Antenatal prophylactic anticoagulation and outcomes of labour and birth management

Kidson-Gerber G[1,2,3], Shand A[2,4], **Curnow J[4,5]**, Gounder P[6,7], El Hamawi Z[2], Piper K[5], Ardui J[6]
[1]Prince of Wales Hospital, Randwick, Australia, [2]Royal Hospital for Women, Randwick, Australia, [3]University of New South Wales, Randwick, Australia, [4]University of Sydney, Camperdown, Australia, [5]Westmead Hospital, Westmead, Australia, [6]Campbelltown Hospital, Campbelltown, Australia, [7]Western Sydney University, Penrith, Australia

Aim: Pregnancy and the puerperium represent periods of increased risk of venous thromboembolism (VTE). Many studies have examined the safety and efficacy of prophylactic anticoagulation to prevent VTE. There are real or perceived bleeding risks with administration of anaesthesia and birth in these women. This retrospective audit examines management of labour, birth and peripartum anaesthesia in women receiving antenatal prophylactic anticoagulation.

Method: In three high-volume metropolitan obstetric units pregnancies were identified in which (1) antenatal prophylactic anticoagulation was prescribed, (2) birth occurred between July 2009 and July 2022 at ≥ 20 weeks gestation. . Data on maternal demographics, pregnancy and birth management was entered into a REDCap database.

Results: 129 pregnancies in 122 women were identified, with median maternal age of 33.2 years, and BMI of 27.3 kg/m2. Indications for anticoagulation are in figure 1. 98.4% of women received enoxaparin. Birth was by spontaneous vaginal birth (26.4%), vaginal birth after induction of labour (34.1%), elective caesarean section (CS) (34.1%) or CS after induction of labour (5.4%), at median 39+0 weeks gestational age. Postpartum haemorrhage occurred in 3.8% of vaginal births and 5.9% of CS. Transfusion was required after 2 births (1.6%).

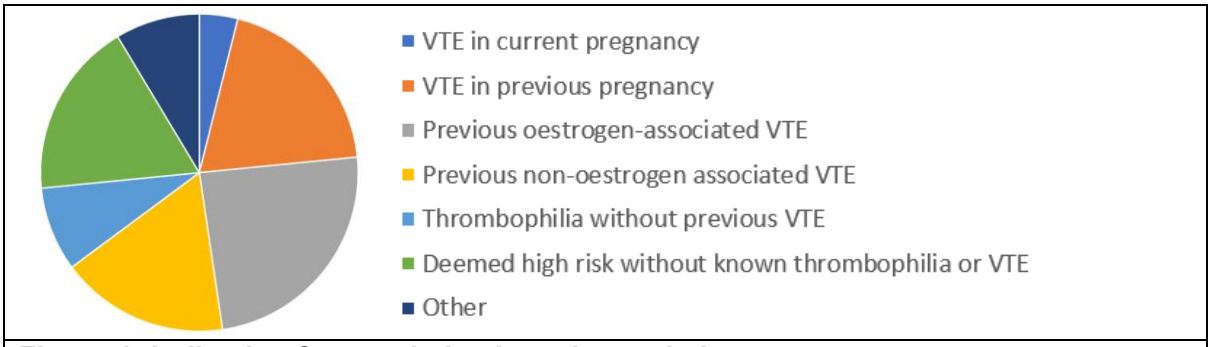


Figure 1: Indication for prophylactic anticoagulation

Anticoagulation was documented as an indication for induction in 20 births (39.2%) and elective CS in 2 births (7.7%). Neuraxial anaesthesia was used in 63 births (48.8%), including 23 vaginal births (29.5% of vaginal births), but was reported to be delayed/declined in 5 births (3.9%), although documentation of decision making was sparse.

Conclusion: 23 women (29.5%) were able to safely have vaginal birth without exclusion of neuraxial anaesthesia. Nonetheless, a large proportion of women receiving prophylactic anticoagulation in our series had scheduled births, with anticoagulation identified as a key indication for induction or elective CS.

Does the presence of heparin in nasopharyngeal swabs interfere with detection of SARS-CoV-2 by PCR? If so, can the effect be prevented through the addition of the heparinase I enzyme?

Edwards K[1,2], Corocher T[1], Hersusianto Y[1,3], Campbell D[4,5], Ho P[1,2,5], Monagle P[6,7,8,9]
[1]Northern Pathology Victoria, Northern Health, Epping, Australia, [2]Northern Clinical Pathology, Thrombosis, And Radiology (NECTAR) Research Group, Northern Health, Epping, Australia, [3]Infectious Diseases, Northern Health, Epping, Australia, [4]Hospital without Walls, Northern Health, Epping, Australia, [5]Department of Medicine, University of Melbourne, Melbourne, Australia, [6]Department of Paediatrics, University of Melbourne, Melbourne, Australia, [7]Haematology Research, Murdoch Children's Research Institute, Parkville, Australia, [8]Clinical Haematology, Royal Children's Hospital, Parkville, Australia, [9]Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia

Aim: Intranasal Heparin is hypothesized to inhibit SARS CoV- 2 infection. This is being investigated in the IntraNasal HEpaRin Trial (INHERIT). However, heparin may interfere with the polymerase chain reaction (PCR), due to interactions with RNA and RNA polymerase. We aimed to investigate how heparin affected various SARS-CoV-2 PCR assays, and if such interference could be ameliorated through the addition of the heparinase I enzyme.

Method: Residual SARS-CoV-2 positive samples were spiked with heparin in concentrations ranging from 10-5000 IU/mL, and SARS-CoV-2 assays performed on the Hologic Panther, GeneXpert, LIAT, Seegene + StarMag, and Seegene + TANBead platforms. The same method was used with the addition of heparinase I (10-250 IU/mL), and assayed on the Seegene + StarMag platform.

Results: The presence of heparin caused interference, however the degree of interference differed between testing platforms. When comparing expected vs. actual results, the percent (%) concurrence rates were: Hologic Panther 100.0%, GeneXpert 40.0%, LIAT 60.4%, Seegene + StarMag 12.5%, and Seegene + TANBead 41.7%.

The addition of heparinase I reversed PCR inhibition on the Seegene + StarMag platform, with resulting CT (cycle threshold) values comparable to those of the sample only controls. In addition, heparinase I was able to almost completely reverse PCR inhibition caused by heparin across a variety of grouped CT values (<20, 20-30, 30-35). In single or double gene positives, borderline incomplete reversal meant that positive/negative decisions may differ.

Conclusion: Overall, these results give confidence that the use of intranasal heparin does not affect the accuracy of SARS-CoV-2 assays when performed on the Hologic Panther platform. In addition, we have identified that utilising the heparinase I enzyme is a valid method for overcoming heparin interference when utilising PCR platforms such as the Seegene StarMag.

Reviewing thrombophilia screen ordering practices following the implementation of a local guideline at a regional hospital in New South Wales

Elton G[1], Zhang L[1,2], Tang C[1]
[1]*Central Coast Local Health District, Gosford, Australia,* [2]*Northern Sydney Local Health District, Sydney , Australia*

Aim: To evaluate the effect of introducing a local guideline paired with junior doctor education on thrombophilia screen ordering practices and to identify further areas where uptake of appropriate clinical practices is poor.

Method: We reviewed data from a 2021 thrombophilia screen audit completed at the same regional hospital to identify areas where non-evidence based practices were being employed. We reviewed current evidence and international guidelines including British Journal of Haematology and Haemostasis Society of Australia and New Zealand to create a local guideline for thrombophilia screen ordering within our local health district. This was approved by the stakeholders and implemented throughout the hospital, paired with education for junior doctors. Doctors who attended teaching completed a survey and an audit was completed on ordering practices by retrospectively reviewing all thrombophilia tests performed on inpatients at Gosford and Wyong Hospital from June to August 2023. The electronic clinical record was reviewed to determine indication for the test, whether haematology was involved and if this led to a change in management. We determined the appropriateness of these tests by analysing them with the local guideline and compared this data to the previous audit to determine if a change in practice was achieved with the implementation of the guideline.

Results: The audit period is not yet complete, however, preliminary qualitative data from surveys suggest that a change in ordering practice will ensue as it showed that junior doctors in this health district have a poor understanding of appropriate indications for testing.

Conclusion: Inpatient screening for thrombophilia's is often not indicated and the implementation of a local guideline paired with education efforts will reduce patient harms and improve stewardship of resources.

Von Willebrand's disease family post DDAVP trial - A laboratory case study

Freeman A[1], Vongkhamchanh J[1]
[1]*Sullivan Nicolaides Pathology, Shailer Park, Australia*

Aim: Von Willebrand's disease is the most common inherited bleeding disorder. From an interesting case study from the laboratory, the effects of DDAVP(Desmopressin) on a family with VWD will be explored to demonstrate the variability of treatment.

Method: Von Willebrand's disease consists of three primary types defined by qualitative or quantitative defects. DDAVP is a drug used primarily for Type 1 Von Willebrand's disease and mild Haemophilia A by stimulating an increase of Factor VIII and VWF into circulation mobilized from endothelial cells. Type 1 VWD responds well to DDAVP as there is a mild reduction of VWF which can be increased from this storage pool. A mother and her 5 children all present with Von Willebrand's disease testing for a DDAVP trial. Von Willebrand factor screen along with collagen binding assay was performed using patient plasma collected pre-DDAVP along with a post 2 hours and post 4-hour DDAVP sample.

Results: All five members of the same family showed differing responses to DDAVP. While VWF antigen, activity, and Factor VIII levels all appear to be increased 2 hours post-administration, the 4-hour post-dose levels showed a reduction in levels apart from one member which did not have a 2-hour post dose to compare. Ristocetin Cofactor and CBA levels followed similar patterns, however, for some members the DDAVP showed little to no effect.

Conclusion: With the data provided from laboratory testing, the DDAVP trial shows increased levels of Factor VIII and VWF antigen levels as expected from the drug. This case study gives data on possible drug effects and variability between patients in the same family showing why the trial is important to provide effective treatment for patients.

No conflict of interest to disclose.

Waldenström Macroglobulinaemia lymphoma patients have impaired platelet haemostatic function

Brysland S[1], Crispin P[2], Talaulikar D[1,3], **Gardiner E[1]**
[1]The John Curtin School of Medical Research, The Australian National University, Canberra, Australia, [2]Department of Clinical Haematology, The Canberra Hospital, Canberra, Australia, [3]School of Medicine and Psychology, The Australian National University, Canberra, Australia

Background: Waldenström Macroglobulinaemia (WM) is a B-cell lymphoma with clinical features including thrombocytopenia, IgM-mediated hyperviscosity and bleeding. Treatment with standard therapies can exacerbate bleeding risk. Abnormal haemostasis (platelet dysfunction and altered coagulation) may result from elevated IgM paraprotein levels.

Objective: To evaluate haemostatic and platelet dysfunction in WM patients and assess the effect of paraprotein inclusion.

Methods: Platelet receptor levels in samples from 19 clinically-annotated WM patients or healthy donors (HD) were measured by flow cytometry. IgM from plasma isolated from a WM patient undergoing plasmapheresis was precipitated using NH_4SO_4 and purified by chromatography on Affigel-Blue resin. Thrombin generation in WM plasma ± platelets and whole blood clotting potential were evaluated in presence/absence of IgM. Platelet aggregation was measured by optical density. Rate and extent of WM or HD platelet spreading was quantified using confocal microscopy and ImageJ software. Soluble GPVI (sGPVI) and thrombopoietin (TPO) levels were measured by ELISA.

Results: WM platelets had reduced levels of GPIbα ($p=0.0153$), GPVI ($p=0.0347$) and reticulation ($p=0.0005$), and increased overall sialylation ($p=0.0079$) and tetraspanin CD9 ($p<0.0001$). WM plasma displayed significantly reduced thrombin generation potential ($p=0.0408$) and WM platelets contributed less to thrombus initiation ($p=0.0208$) and rate ($p<0.0001$) by ROTEM, but bound fibrinogen in response to standard agonists normally. Plasma sGPVI was within normal ranges; TPO levels were increased ($p<0.0001$). HD platelets, mixed with 30-60 mg/mL patient-derived IgM but not control protein molar equivalents, had slower and diminished spreading, aggregation, thrombin generation and thrombus formation.

Conclusions: Alterations to thrombin potential, whole blood coagulation and platelet properties and function, which were exacerbated by inclusion of patient IgM at sub-hyperviscous and hyperviscous concentrations, may underpin bleeding in WM. Monitoring platelet and coagulation properties may help stratify WM patients for bleeding risk.

Bleeding severity and haemostatic management of inherited Rare Bleeding Disorders (RBDs) at an Australian Haemophilia Treatment Centre.

Grainger B[1], Davis A[1], McFadyen J[1,2,3,4], Tran H[1,2]
[1]Department of Clinical Haematology, The Alfred Hospital, Melbourne, Australia, [2]Australian Centre for Blood Diseases, Central Clinical School, Monash University, Melbourne, Australia, [3]Atherothrombosis and Vascular Biology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia, [4]Baker Department of Cardiometabolic Health, University of Melbourne, Melbourne, Australia

Aim: Rare bleeding disorders (RBDs) refer to inherited deficiencies of coagulation factors other than VIII and IX. We sought to investigate the relationship between factor activity level and bleeding severity in patients with RBDs at a large Australian Haemophilia Treatment Centre (HTC).

Method: A cross-sectional analysis was performed of patients registered in the Australian Bleeding Disorders Registry (ABDR) at the Alfred Hospital HTC with factor V, VII, X, XI or XIII deficiency as of March 31, 2023. Fibrinogen disorders are currently the subject of a separate project at our institution and so were excluded. Bleeding episodes were retrospectively assessed using both the International Society of Thrombosis and Haemostasis bleeding assessment tool (ISTH-BAT) and European Network of Rare Bleeding Disorders (EN-RBD) severity category. Linear regression analysis was used to determine the association between factor activity level (dependent variable) versus ISTH-BAT and EN-RBD grade (independent variables).

Results: 61 patients had adequate records for analysis as tabulated below:

	FV (n = 4)	**FVII (n = 19)**	**FX (n = 4)**	**FXI (n = 28)**	**FXIII (n = 6)**
Age	38 (28-33)	52 (30-62)	34 (29-36)	54 (41-79)	31 (27-32)
Male (%)	25	63	50	54	83
Baseline factor activity	2 (2-11)	11 (5-29)	12 (1-26)	2.5 (1-6)	8.5 (2.5-10)
ISTH-BAT score	5 (4-7)	3 (0-4)	3 (2-5)	3 (1-6)	7 (6-11)
EN-RBD grade (%) No bleeding Grade 3 (severe)	 25 25	 42 16	 0 25	 29 2	 0 17
ISTH-BAT vs factor activity (r^2)	0.5525	0.1597	0.5717	0.0187	0.3447
EN-RBD vs factor activity (r^2)	0.8596	0.0487	0.2173	0.0112	0.4402
Peri-procedural prophylactic haemostatic agent use (%)	25	31	25	43	20†

All data are median (IQR), unless otherwise stated.
† 5 of the 6 patients with FXIII deficiency were already receiving regular prophylactic FXIII concentrate

Conclusion: The relationship between factor activity and bleeding severity by both tools was strongest for FV, FX and FXIII and weakest for FXI, similar to previous reports. Prophylactic use of FXI concentrate is common, despite FXI deficiency having the mildest bleeding phenotype at presentation and weakest correlation between factor activity and bleeding.

Treatment of bleeding episodes with efanesoctocog alfa in patients with severe haemophilia A in the phase 3 XTEND-1 study

Khoo L[1], Weyand A[2], Meunier S[3], Amano K[4], Bystricka L[5], Neill G[6], Abad-Franch L[7], Willemze A[8], Tosetto A[9]

[1]Institute of Haematology, Royal Prince Alfred Hospital, Sydney, Australia, [2]Division of Hematology/Oncology, Department of Pediatrics, University of Michigan, Ann Arbor, USA, [3]Hospices Civils de Lyon, Groupement Hospitalo Universitaire Est, Unité Hémostase Clinique, Bron, France, [4]Department of Laboratory Medicine, Tokyo Medical University, Tokyo, Japan, [5]Sobi, Stockholm, Sweden, [6]Sanofi, Reading, UK, [7]Sobi, Basel, Switzerland, [8]Sanofi, Amsterdam, Netherlands, [9]Hemophilia and Thrombosis Center, Hematology Department, San Bortolo Hospital, Vicenza, Italy

Aim: The Phase 3 study XTEND-1 (NCT04161495) demonstrated that once-weekly efanesoctocog alfa provided superior bleed prevention to prior FVIII prophylaxis and was well tolerated in adults/adolescents with severe hemophilia A. Here, we report on efficacy of efanesoctocog alfa for treatment of bleeds during XTEND-1.

Methods: Patients on prior prophylaxis entered Arm A (52 weeks once-weekly efanesoctocog alfa [50 IU/kg]). Patients receiving prior on-demand therapy entered Arm B (26 weeks on-demand efanesoctocog alfa [50 IU/kg], then 26 weeks once-weekly prophylaxis). Bleeds were to be treated with a single efanesoctocog alfa 50 IU/kg dose with additional doses (30 or 50 IU/kg every 2–3 days) if needed. Number and location of treated bleeds, required dose/number of efanesoctocog alfa injections for bleed resolution, and response to bleed treatment were evaluated.

Results: Median (range) annualized bleed rate was 0.0 (0.0–11.0) in Arm A (n=133), and 21.1 (8.3–33.4) and 0.0 (0.0–4.1) for Arm B on-demand and prophylaxis periods (n=26), respectively. Of 362 bleeds treated with efanesoctocog alfa, 86 occurred in Arm A, 268 during Arm B on-demand treatment, and 8 during Arm B prophylaxis. In Arm A, 33 (38%) bleeds were spontaneous, 45 (52%) traumatic, and 8 (9%) of unknown aetiology. Corresponding Arm B values were 197 (74%), 62 (23%), and 9 (3%) for the on-demand period, and 5 (63%), 2 (25%), and 1 (13%) for the prophylaxis period. Most bleeds occurred in joints (79%) and muscles (14%). Most (97%) of bleeds were treated with a single injection, and all but 1 resolved with ≤2 injections. Median (interquartile range) total dose for bleed treatment was 50.9 (50.0–51.8) IU/kg. Most (95%) responses to bleed treatment were excellent/good.

Conclusion: A single efanesoctocog alfa 50 IU/kg dose effectively treated 97% of bleeds regardless of bleed type and location in adults/adolescents with severe hemophilia A.

Paediatric APTT reference intervals using Triniclot APTT S and Triniclot APTT HS reagents in combination with STA line instrument.

Letunica N[1], Karlaftis V[1,2], Monagle P[1,2,3], Stephenson-Brown A[4], Guppy V[4]
[1]Haematology, Murdoch Children's Research Institute, Melbourne, Australia, [2]Department of Paediatrics, The University of Melbourne, Melbourne, Australia, [3]Department of Clinical Haematology, Royal Children's Hospital, Melbourne, Australia, [4]Stago ANZ , Forrest Hill, Australia

Aim: Previous studies in the setting of developmental haemostasis have established age-specific differences in the quantity, function of haemostatic proteins and functional assays. Routine and specialized haemostasis assays in the paediatric population should thus be undertaken with these differences in mind. Reagent, analyser and age-specific reference ranges should be established for all clinically utilised laboratory tests. This study aimed to establish age-related reference intervals for activated partial thromboplastin time (APTT), using the STA R Max® analyser (Diagnostica Stago, France) and reagents Triniclot APTT S and Triniclot APTT HS from Stago group company TCoag (TCoag, Ireland).

Methods: Citrated plasma samples were obtained via clean venepuncture from healthy neonates and healthy children undergoing elective surgery (e.g. circumcision). Results were divided into the following age groups: newborns (from birth to 1 month of age), infants (greater than 1 month to 2 years of age), children (greater than 2 to 12 years of age) and adolescents (greater than 12 to 18 years of age). Results were analysed using R version 4.1.1 and are expressed as mean with intervals including 95% of the population (2.5th percentile - 97.5th percentile) in accordance with CLSI guidelines.

Results: Mean values and reference intervals according to age are presented in Table 1. The mean values for both APTT S and APTT HS differ significantly between newborns and infants when compared to adolescents.

Conclusion: This study confirms the need to develop analyser and reagent specific reference intervals across the age spectrum, enabling accurate interpretation of coagulation tests and accurate diagnosis for newborns, infants and children.

Table 1: Reference intervals for neonates, infants, children and adolescents.

	Newborns Birth to 1 month	**Infants >1 Month-2 Years**	**Children >2 Years-12 Years**	**Adolescents >12 Years-18 Years**
Triniclot APTT S (sec)	n=20 *	n= 12 *	n= 26	n= 22
Mean	45.4	48.4	37.4	38.2
Reference interval	42.4 - 48.4	42.3 - 54.5	35.0 - 39.9	36.1 - 40.3
Triniclot APTT HS (sec)	n= 20*	n= 12 *	n= 26	n=22
Mean	42.2|	47.9	35.9	36.1
Reference interval	39.3 - 45.0	41.6 - 54.2	33.9 - 37.9	34.2 - 38.0

* P-value significantly different from adolescents (p=<0.05)

A 12-year review of upper extremity deep vein thrombosis – are they the same as lower extremity deep vein thrombosis?

Leung P[1], Lui B[1], Ho P[1], Lim H[1]
[1]*Northern Health, Epping, Australia*

Aim: Upper extremity deep vein thrombosis (UE-DVT), defined as thrombosis of the upper extremity and/or thoracic inlet, accounts for approximately 5% of DVTs[1,2]. Optimal treatment of UE-DVTs is yet to be determined. Here, we examined the epidemiology and treatment outcomes of UE-DVT.

Method: A retrospective review was conducted on patients diagnosed with UE-DVT between December 2010 and December 2022 at Northern Health, Victoria, Australia. Medical records were reviewed to confirm diagnosis and assess treatment outcomes.

Results: 138 patients with UE-DVT were identified (52.2% females; median age 61.5 years). Nearly all patients (92.8%) were symptomatic at time of diagnosis. Ten patients (7.2%) had a concurrent diagnosis of pulmonary embolism. 114 patients (82.6%) had at least one clear provoking factor, the most common being malignancy (43.9%) and catheter-associated (53.5%). Fourteen patients (10.1%) were subsequently diagnosed with thoracic outlet syndrome/Paget-Schroetter syndrome, of which eight received endovascular or surgical intervention. 111 patients (80.4%) received limited duration therapeutic anticoagulation (median 3 months) with enoxaparin the most common acute anticoagulant of choice. 75/83 (90.4%) patients with repeat imaging demonstrated complete resolution or reduction in clot burden (average time to imaging 115.5 days). Seven patients had non-fatal major bleeding whilst on therapeutic anticoagulation (1.84/100-patient-years). Eight patients developed clot progression while on therapeutic anticoagulation (2.10/100-patient-years) and 11 had recurrent VTE post anticoagulation cessation (2.89/100-patient-years), with no significant difference to the rate of major bleeding (p=0.25). Five patients reported symptoms suggestive of post thrombotic syndrome. There were 19 deaths (13.8%), of which one was partially attributed to clot extension.

Conclusion: Malignancies and/or venous catheters are the most common causes of UE-DVT with 10% of patients identified to have Paget-Schroetter syndrome. The VTE recurrence rate of 2.89/100-patient-years is not insignificant and appropriate VTE risk assessment should be considered when making treatment decisions. Interventional techniques may be appropriate for select patients.

References:

- Cote LP, Greenberg S, Caprini JA, et al. Comparisons Between Upper and Lower Extremity Deep Vein Thrombosis: A Review of the RIETE Registry. Clin Appl Thromb Hemost. 2017;23(7):748-754.

1. Ageno W, Haas S, Weitz JI, et al. Characteristics and Management of Patients with Venous Thromboembolism: The GARFIELD-VTE Registry. Thromb Haemost. 2019;119(2):319-327.

Treatment and outcomes of cancer-associated thrombosis, from traditional anticoagulation options to direct oral anticoagulants: a ten-year experience

Wee B[1], Lui B[1], Ho P[1,2], **Lim H[1,2]**

[1]Northern Clinical Pathology, Thrombosis and Radiology (NECTAR) Research Group, Northern Health, Epping, Australia, [2]Department of Medicine (Northern Health), University of Melbourne, Heidelberg, Australia

Aim: Direct oral anticoagulants (DOACs) have emerged as first-line treatment in most cancer-associated thrombosis (CAT), representing a paradigm shift in its management. However, the management of CAT remains challenging and requires careful risk-benefit considerations for individual patients.

This study analysed the shift of CAT management to DOACs over the last decade and its impact on real-world clinical outcomes.

Method: A retrospective analysis of CAT presentations to a tertiary referral centre from January 2011 to December 2020. The outcomes in CAT patients were compared with venous thromboembolism (VTE) patients without malignancy. A subgroup analysis was also conducted for CAT patients according to the type of anticoagulation.

Results: A total of 512 cases of CAT from 489 patients were identified from 3230 total VTE cases.

CAT patients had higher rates of major VTE (PE and/or proximal DVT) compared to patients without malignancy (78.5% vs. 66.7%, p<0.001). CAT patients also had higher rates of VTE recurrence (HR 1.81, 95% CI 1.33 – 2.45), major bleeding (HR 2.77, 95% CI 1.78 – 4.31), VTE-related mortality (HR 2.54, 95% CI 1.40 – 4.59) and bleeding-related mortality (HR 2.66, 95% CI 1.05 – 6.73).

When comparing CAT patients treated with DOACs to those treated with enoxaparin or warfarin, there were no significant differences in rates of VTE recurrence, major bleeding, VTE-related mortality or fatal bleeding. In the subgroup of CAT treated with DOACs, there was no significant difference in rates of GI bleeding compared to the enoxaparin subgroup (3.2% vs. 4.2%, p=0.774).

Conclusion: CAT was associated with a larger clot burden and higher rates of VTE recurrence, major bleeding and mortality compared to VTE patients without malignancy. There were no significant differences in complication rates for CAT patients treated with DOACs over enoxaparin, suggesting that DOACs can be safely used in most cases of CAT.

Retrospective study of clinical settings, indications and consequences of measurement of Direct Oral Anticoagulant plasma levels in Northern Tasmania, Australia

Lim M[1,2], Morse M[1,2], Gunson H[3], Mohamed M[1,2,4]
[1]Launceston General Hospital, Launceston, Tasmania, Australia, Australia, [2]Northern Tasmanian Pathology Service, Launceston, Australia, [3]Pathology South, Royal Hobart Hospital, Hobart, Australia, [4]University of Tasmania, Launceston, Australia

Aim: To evaluate the clinical settings, indications and changes to anticoagulant management associated with DOAC level measurement in a tertiary hospital in Tasmania, Australia

Method: Patients with at least one DOAC level (dabigatran, rivaroxaban or apixaban) requested between Jan 2017 and Dec 2022 were identified using the haematology laboratory database. Anti-Xa and dilute thrombin time were performed for measurement of rivaroxaban/apixaban and dabigatran levels respectively. Retrospective chart review was performed to evaluate the clinical settings, indications, adequacy of information provided on request form, and changes to clinical management associated with measurement of DOAC levels.

Results: A total of 129 DOAC levels (54 rivaroxaban, 66 apixaban, 9 dabigatran) performed on 98 patients were requested between January 2017 to December 2022 (after exclusion of DOAC levels requested for research purposes only in a separate study and requests with no clinical information on the electronic medical record). Annual requests for DOAC levels increased significantly from 2018 to 2019 with a more than 3-fold increase followed by a decline in 2022. Overall, the most common indication for a DOAC level (including cases with ≥2 indications) was for renal impairment, followed by bleeding and recurrent thrombosis. Approximately 1 in 4 requests were for acute bleeding with a reversal/pro-haemostatic agent given in 60% of these cases while 1 in 10 were prior to urgent surgery. Measurement of DOAC levels was associated with a change in management in 50% of cases. 1 in 10 requests did not specify anticoagulant history.

Conclusion: Requests for DOAC levels have increased over time and may be useful in certain clinical settings. Education of clinicians of the importance of providing information regarding specific anticoagulant history is essential to ensure the appropriate DOAC assay is performed by the laboratory. Future prospective studies investigating the clinical utility of DOAC levels prior to surgery or administration of reversal/pro-haemostatic agents in acute major bleeding are needed.

Retrospective review of VTE events in orthopaedic patients with lower limb immobilisation

Lin C[1], Bortz H[1]
[1]*Alfred Health, Melbourne, Australia*

Aim: To review the use of antithrombotic prophylaxis and identify the incidence of venous thromboembolism (VTE) in orthopaedic patients following lower limb (LL) immobilisation.

Method: Single-centre retrospective study conducted at Alfred Health, a quaternary referral centre. Electronic medical records of orthopaedic patients discharged during the three-month period February-May 2022 were reviewed. Patients >18 years old who left hospital with LL immobilisation following injury and/or surgery were included. Patients who had hip/knee arthroplasty, were premorbidly anticoagulated, or a length of stay <24 hours were excluded. For 133 included patients, data on inpatient VTE prophylaxis, delay/interruption to prophylaxis, discharge antithrombotic agent, clinical outcomes (VTE, bleeding), ICU admission, rehospitalisation and all-cause mortality were collected. Follow-up period was 30 days post-discharge. Subgroup analyses were performed using GraphPad Prism®. A p-value <0.05 was considered statistically significant.

Results: Overall, the median age was 46 years, 57% of patients were male, and 17% had VTE risk factors. Foot/ankle injuries were the most common reason for immobilisation (n=55, 41%). Inpatient VTE prophylaxis was prescribed in 91% of patients, and 72.2% were prescribed an antithrombotic agent on discharge, with aspirin being the most common (43/96, 45.1%). VTE incidence was 3% (4/133). Subgroup analysis demonstrated a higher rate of VTE in patients with delay/interruption to inpatient thromboprophylaxis compared to those without (6% vs. 0%; p=0.04). There was one case of major bleeding (0.75%). Incidence of rehospitalisation was 9.8%, there was one ICU admission and no deaths.

Conclusion: There was a low incidence of VTE events in orthopaedic patients with LL immobilisation, with rates comparing favourably with existing literature, despite almost one-third of our study population receiving no antithrombotic prophylaxis on discharge. Marked variation remains in choice of thromboprophylaxis following LL immobilisation and the importance of clinical judgement in this domain is recognised in current guidelines.

Case Study of a haemostatic approach for an AVR with ascending aorta and aortic arch replacement utilising cardiac bypass in a Jehovah's Witness.

Lombard M[1,2], Le Viellez A[2], Larbalestier R[1], Coombs L[1], Devine G[1], Ross J[1], P'Ng S[1,2]
[1]Fiona Stanley Hospital, Murdoch, Australia, [2]Pathwest, Murdoch, Australia

Background: Jehovah's Witness patients refuse transfusion of blood products based on biblical teaching. Cardiac surgery procedures involving cardiac bypass necessitate blood product transfusion (25-95%) (1-3). Cardiac surgery represents significant challenge in Jehovah's Witness patients requiring multi-disciplinary input from Anaesthetics, Haematologists, Cardiologists and Surgeons.

Method: A 63 year old, 98kg female with a normal coagulation profile, was admitted with symptomatic critical aortic stenosis (peak velocity 6m/s, mean gradient 100mmHg, preserved LV function), and thoracic aortic dilatation (ascending aorta 4.9cm and proximal aortic arch 5.2cm). She identified as a Jehovah's witness and after consulting with her church adviser was unaccepting of red blood cell transfusions, platelets, FFP or cryoprecipitate but accepting of Factor Concentrate (Prothrombinex), Factor VIII concentrate (Biostate), Fibrinogen Concentrate (Riastap), DDAVP, Tranexamic acid and albumin for haemostasis.

Haemostatic Approach: The patient was given an iron infusion 7 days prior to procedure and perioperatively received 2000IU Prothrombinex, 2000IU Factor VIII concentrate, 2g Fibrinogen Concentrate, DDAVP 28 micrograms and intraoperative Tranexamic acid. Cell salvage was used intraoperatively.

Outcome: The patient had a successful aortic valve replacement, aortic arch replacement and debranching of the innominate and left carotid arteries. A bypass with priming of albumin, heparin and plasmalyte, had a total time of 218 minutes with 173 minutes of cross clamp time and 13 minutes of cardiac arrest. End of procedure protamine was given at the conclusion of the procedure. There was 200mls frank haematuria during the procedure. The patient was transferred to ICU for 48 hours monitoring post-operatively and had an INR of 1.3 with a PT of 18.6. The pre-surgical haemoglobin was 139 and post-operative was 101. The patient was commenced on routine heparin and warfarin 24 hours post-operatively. The post-operative course was complicated by pericarditis with discharge on day 7 post-operatively. At 30-days there were no bleeding or clotting events.

A case of LA-HPS

Lovelock D[1], Hui E[1], Adams R[1]
[1]*Sullivan Nicolaides Pathology, Bowen Hills, Australia*

Aim: Lupus anticoagulant-hypoprothrombinemia syndrome (LA-HPS) is an extremely rare coagulation disorder. This case study will review the pathophysiology, challenges in laboratory diagnosis and treatment of this disorder.

Method: Retrospective case study of an individual patient presenting with a bleeding diathesis **and subsequent diagnosis of LA-HPS.**

Results: A 4 year old boy presented to emergency with haematuria and bruising. The patient had a prolonged prothrombin time (PT) of 38.4 seconds (s) (RR 10–14s) and activated partial thromboplastin time (APTT) of 106.2s (RR 24–38s). The patient had no personal or familial history of a bleeding disorder. Of note, the entire family had experienced a diarrhoeal illness one week prior to presentation. The citrate sample was referred for specialist coagulation testing, which included coagulation factor assays and lupus anticoagulant (LA) screening. Factor II was markedly decreased (<0.01U/mL; RR 0.5-2.0U/mL), and APTT showed no correction on mixing studies. , Lupus anticoagulant testing confirmed the presence of a lupus anticoagulant. These findings were most suggestive of LA-HPS. This rare syndrome is associated with auto immune diseases, such as systemic lupus erythematosus, as well as infectious causes. Whereas lupus anticoagulant is usually associated with an increased risk of thrombosis, in contrast, LA-HPS often presents with a bleeding phenotype.

Conclusion: LA-HPS is a rare disorder, where patients can present with haemorrhagic complications rather than thrombosis. Laboratory diagnosis can be challenging, however prompt diagnosis is necessary for appropriate patient care

No conflicts of interest to disclose.

Real world experience of Emicizumab use in Acquired Haemophilia A

Mason J[1,2,3], Campbell S[1,3]
[1]Qld Haemophilia Centre, Royal Brisbane and Women's Hospital, Herston, Australia, [2]Pathology QLD, Herston, Australia, [3]UQ School of Medicine, Brisbane, Australia

Introduction: Acquired haemophilia A (AHA) is a rare potentially life-threatening disease caused by the development of autoantibodies against factor VIII (FVIII). Small retrospective case series and preliminary data from a prospective study suggest that there is early haemostatic effect within days of commencing Emicizumab in AHA patients, with cessation of bleeding or lack of development of new bleeding, ability to cease bypassing agents and earlier discharge. There is limited real-world experience with the use of this agent for AHA.

Aim: To characterise clinicopathologic features and outcomes in a series of patients with AHA who received Emicizumab at the QLD Haemophilia Centre. Dosing protocols, clinical efficacy and tolerability of Emicizumab will be explored. Usage of bypassing agents and immunosuppressive therapy (IST) will be compared to a historic (previously published) local cohort.

Method: A retrospective review of all patients with AHA receiving Emicizumab at the QLD Haemophilia Centre over a 2-year period.

Results: Seven patients were identified (age range 19-67 years). Median FVIII was <1% (range <1% to 3%) and inhibitor titre 234.9 BU (range 11.9 BU to 870 BU). Bleeding was severe in six patients and non-severe in one. All patients received IST with a prednisone backbone and the addition of low dose Rituximab in six patients. Emicizumab dosing was not uniform. Six patients had no new bleeding after Emicizumab initiation, one had a minor non-progressive bleed. Three patients required no rFVIIa after the first dose of Emicizumab and no patients required further bypassing agents after the second dose.

Conclusion: Emicizumab was efficacious in our series with no serious side effects. Use of bypassing agents was reduced compared to the historic cohort. Exploration of dedicated rapid AHA Emicizumab loading protocols may further reduce bleeding, need for bypassing agents and allow less intensive IST.

Durability of bleeding protection and factor IX activity in those with and without AAV5 neutralising antibodies in the phase 3 HOPE-B trial of etranacogene dezaparvovec for haemophilia B

Monahan P[1], Pipe S[2], Leebeek F[3], Recht M[4], Key N[5], Lattimore S[9], Castaman G[6], Cooper D[7], Verweij S[7], Dolmetsch R[7], Tarrant J[1], Li Y[1], Miesbach W[8]
[1]CSL Behring, King of Prussia, USA, [2]University of Michigan, Ann Arbor, USA, [3]Erasmus MC, University Medical Center, Rotterdam, The Netherlands, [4]Yale University School of Medicine, New Haven, USA, [5]University of North Carolina, Chapel Hill, USA, [6]Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy, [7]uniQure BV & uniQure Inc , Amsterdam & Lexington, MA, The Netherlands & USA, [8]University Hospital Frankfurt, Frankfurt, Germany, [9]Oregon Health & Science University, Portland, USA

Aim: Adeno-associated virus (AAV) neutralising antibodies (NAbs) previously limited the efficacy of AAV-based gene therapy. The phase 3 HOPE-B trial (NCT03569891) assesses etranacogene dezaparvovec, an AAV5 vector expressing Padua factor IX (FIX), in those with (NAb+) and without (NAb-) AAV5 NAbs over 24 months.

Method: Adult males with severe/moderately-severe haemophilia B (FIX ≤2%) received etranacogene dezaparvovec (single dose; $2x10^{13}$ gc/kg) in this international, open-label, single-arm trial after ≥6-months lead-in on FIX prophylaxis. FIX activity, annualised bleed rate (ABR) and FIX product use were assessed during lead-in and after dosing. Adverse events (AEs) were recorded. AAV5 NAbs were assessed on day of dosing (baseline; AAV5 transduction inhibition assay).

Results: Fifty-four participants were dosed (NAb-, n=33; NAb+, n=21 at baseline). Median (Q1–Q3) AAV5 NAb titre in NAb+ participants was 56.9 (23.3–198.9); 20/21 (95%) NAb+ participants had titres <1:700. One participant (titre 3212) did not express FIX Padua and one (titre 198.9) received a partial dose; all others (52/54) discontinued FIX prophylaxis.

At 24 months post-dose, no correlation between baseline NAb titre and FIX level was identified up to titre <1:700 (24-months Pearson's r: -0.29; Spearman's rho: -0.25; R^2: 0.086). FIX levels (median [min–max]) were sustained at 24 months post-dose (NAb+ <1:700: 33.5% [9.1–88.3] and NAb-: 35.4% [4.7–99.2] participants).

NAb+ <1:700 and NAb- participants had low ABRs during Months 7–24 (1.65 and 0.80, respectively). These ABRs were significantly improved vs lead-in (NAb+ <1:700: 4.29 [vs Months 7–24 p=0.0065]; NAb-: 3.80 [vs Months 7–24 p<0.0001]).

AE profiles were similar for NAb subgroups at 24 months (corticosteroid-treated transaminase elevations: 6/33 NAb- [18.2%], 3/21 NAb+ [14.3%]; infusion-related reactions: 2/33 NAb- [6.1%] and 5/21 NAb+ [23.8%]).

Conclusion: Etranacogene dezaparvovec provided significant ABR reductions and an acceptable AE profile, regardless of NAb status, through 24 months' follow-up. There was no association between baseline NAb status (titre <1:700) and long-term durability of FIX activity.

Health-related quality of life in the two years following etranacogene dezaparvovec gene therapy for haemophilia B in the phase 3 HOPE-B trial

Monahan P[1], Recht M[2], Itzler R[1], Pipe S[4]
[1]CSL Behring, King of Prussia, PA 19406-0901, USA, [2]Yale University School of Medicine, New Haven, USA, [3]Everest Clinical Research, Little Falls, USA, [4]University of Michigan, Ann Arbor, USA

Aim: Although good haemostatic protection for haemophilia B may be achieved using current factor IX (FIX) prophylactic regimens, strict adherence is necessary and burdensome. Breakthrough bleeding and arthropathy occur and negatively impact health related quality of life (HRQoL). The ongoing Phase 3 HOPE-B trial evaluates the investigational gene therapy, etranacogene dezaparvovec, with the goal to provide haemostatically effective, consistent and durable expression of FIX Padua following discontinuation of FIX prophylaxis.

Method: Adult men with haemophilia B (FIX≤2%) received standard of care FIX concentrate prophylaxis during a ≥6-month lead-in observation followed by a single etranacogene dezaparvovec administration. HRQoL was assessed with the EQ-5D-5L as a generic instrument and the haemophilia-specific Hem-A-QoL over the lead-in and at 6, 12 and 24 months after etranacogene dezaparvovec using repeated measures linear mixed models with a least square (LS) mean difference between the treatment periods. A one-sided p-value ≤0.025 for the post-treatment to lead-in period was considered statistically significant.

Results: There were no statistically significant improvements in the LS mean EQ-5D-5L scores in the first year after gene therapy. However, in the second year, the EQ-5D-5L Visual Analog Scale (VAS) and Index Score (IS) each demonstrated a nominally statistically significant improvement (VAS: LS mean increase 2.8; p=0.024; IS: LS mean increase 0.044; p=0.013). Consistent improvements were observed using the disease-specific Hem-A-QoL tool in the first and second years after gene therapy for the Total score and 4 of 10 domain scores. Model-based mean improvements in scores and the percent improvement at 24 months compared with lead-in were nominally statistically significant: Total Score (LS mean -6.2; p<0.0001; 23.7%), domains 'Treatment' (LS mean -14.24; p<0.0001; 55.2%), 'Feelings' (LS mean -9.10; p<0.0001; 44.8%), 'Future' (LS mean -6.57; p=0.0004; 21.1%) 'Work/School' (LS mean -5.24; p=0.0102; 30.3%).

Conclusion: Following sustained FIX expression for 24 months after etranacogene dezaparvovec gene therapy, HOPE-B trial participants reported reduced treatment burden and feelings consistent with increased optimism for the future.

Adults with haemophilia B receiving etranacogene dezaparvovec in the HOPE-B phase 3 trial experience a stable increase in mean factor IX activity and durable haemostatic protection after 24 months' follow-up

Monahan P[1], Pipe S[2], Leebeek F[3], Recht M[4], Key N[5], Lattimore S[6], Castaman G[7], Coppens M[8], Cooper D[9], Gut R[9], Slawka S[9], Verweij S[9], Dolmetsch R[9], Li Y[1], Miesbach W[10]
[1]CSL Behring, King of Prussia, USA, [2]University of Michigan, Ann Arbor, USA, [3]Erasmus MC, University Medical Center , Rotterdam, The Netherlands, [4]Yale University, New Haven, USA, [5]University of North Carolina, Chapel Hill, USA, [6]Oregon Health & Science University, Portland, USA, [7]Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy, [8]Department of Vascular Medicine, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam,, Amsterdam, Netherlands, [9]uniQure BV & uniQure Inc, Amsterdam & Lexington, MA, The Netherlands & USA, [10]University Hospital Frankfurt, Frankfurt, Germany

Aim: The Phase 3 HOPE-B trial (NCT03569891) assesses etranacogene dezaparvovec, an investigational gene therapy for haemophilia B comprising an adeno-associated virus 5 (AAV5) vector and codon-optimised factor IX (FIX) Padua R338L transgene with a liver-specific promoter.

Method: In this international, open-label, single-arm study, adult males with severe/moderately-severe haemophilia B (FIX ≤2%), with/without pre-existing AAV5 neutralising antibodies (NAbs), received etranacogene dezaparvovec (single dose; $2x10^{13}$ gc/kg) after a ≥6-month lead-in period on FIX prophylaxis. FIX activity, annualised bleed rate (ABR) and FIX infusions were assessed during lead-in and after receiving etranacogene dezaparvovec. Adverse events (AEs) were recorded.

Results: Of 54 participants dosed, 53 received full dose and 52 completed 24 months' follow-up. At 464 days post-infusion, one 75-year-old participant died from cardiogenic shock, preceded by a urinary tract infection (unrelated to treatment).

Of 54 participants, 52 (96.3%) discontinued and remained free of continuous FIX prophylaxis from Day 21 to Months 7–24, including 20 with baseline AAV5 NAb titres <1:700. One participant with a higher AAV5 NAbs titre (1:3212) and one who received partial dose did not express FIX Padua nor discontinue FIX prophylaxis.

Compared with lead-in (mean ABR 4.18), mean ABR for all bleeds during Months 7–24 post-treatment was significantly reduced by 64% (mean ABR 1.51; p=0.0002). Mean FIX activity was 39.0 IU/dL (standard deviation: ±18.7; min–max: 8.2–97.1) at Month 6 (n=51) and 36.7 IU/dL (±19.0; 4.7–99.2) at Month 24 post-treatment (n=50).

Mean unadjusted annualised FIX consumption reduced by 97% from lead-in to Months 7–24 (257,339 vs 8,946 IU/year/participant; p<0.0001).

Over 24 months post-dose, 38 participants (70.4%) had 93 treatment-related AEs; one occurred after Month 24 (Day 735 post-dosing). There were no treatment-related serious AEs.

Conclusion: After 24 months' follow-up, single-dose etranacogene dezaparvovec resulted in stable FIX Padua expression in participants with AAV5 NAbs undetected or <1:700 titre; reduction in ABR remained durable and superior to FIX prophylaxis.

Durability of response after long-term follow-up in the phase 1/2 study of AMT-060, and Phase 2b and 3 Studies of etranacogene dezaparvovec in haemophilia B

Monahan P[1], Miesbach W[2], Recht M[3], Key N[4], Sivamurthy K[1], Pipe S[5]
[1]CSL Behring, King of Prussia, PA 19406-0901, USA, [2]University Hospital Frankfurt, Frankfurt, Germany, [3]Yale University School of Medicine, New Haven , USA, [4]University of North Carolina, Chapel Hill, USA, [5]University of Michigan, Ann Arbor, USA

Aim: Gene therapy for haemophilia B (HB) has potential for durable responses. We review the durability of AMT-060 (wild-type factor IX [FIX]) and etranacogene dezaparvovec (AMT-061; FIX Padua), defined by sustained FIX activity and haemostatic protection.

Method: Three ongoing trials in adults with severe/moderately-severe HB have data over 5 years (phase 1/2 study of AMT-060, N=10; cohort 1: n=5, $5x10^{12}$ gc/kg; cohort 2: n=5, $2x10^{13}$ gc/kg; NCT02396342), 3 years (phase 2b study of etranacogene dezaparvovec, N=3; $2x10^{13}$ gc/kg; NCT03489291) and 2 years (phase 3 HOPE-B study of etranacogene dezaparvovec, N=54; $2x10^{13}$ gc/kg; NCT03569891).

Results: In the phase 1/2 AMT-060 study, mean FIX activity remained stable 5 years post-dose (cohort 1, 52 weeks: 4.4%, Year 5: 5.2%; cohort 2, 26 weeks: 6.9%, Year 5: 7.4%). Mean FIX activity was greater with etranacogene dezaparvovec and remained in the near-normal range after 3 (phase 2b: 36.9%) and 2 years' (phase 3: 36.7%) follow-up. In the Phase 2b study, mean FIX activity increased from Week 3 (23.4%; n=3) to Year 3 (36.9%; n=2); in Phase 3, mean FIX activity was sustained (Month 6: 39.0%, n=51; Year 2: 36.7%, n=50).

In the phase 1/2 AMT-060 study, decreases in mean annualised bleeding rate (ABR) were maintained over 5 years. Similar low ABRs were seen with etranacogene dezaparvovec. In phase 2b, ABR over 3 years was 0.22. In the phase 3 study, ABR (all bleeds) at Months 7–24 was 1.51 (reduced from 4.18 at baseline; full analysis set, n=54). Similarly, ABR reduced from 4.00 at baseline to 0.95 at Months 7–24 in the modified intent-to-treat population (that excluded two non-responders; n=52).

No new safety signals were identified.

Conclusion: Gene therapy for HB appears to have a durable response. In the Phase 1/2 study of AMT-060 and Phase 2b and 3 studies of etranacogene dezaparvovec, FIX activity was sustained and reductions in bleeding events remained stable.

Does protein S deficiency occur in the nephrotic syndrome? A systematic review

Murton A[1,2], Goodall E[1,2], Chunilal S[1,2]
[1]*Monash Haematology, Monash Health, Melbourne, Australia,* [2]*Monash University, Melbourne, Australia*

Venous thromboembolism (VTE) occurs in 20-50% of patients with nephrotic syndrome[1], with numerous implicated mechanisms. Protein S deficiency has been reported as a contributing factor[2,3], however the prevalence of free protein S deficiency in patients with nephrotic syndrome is unknown.

Aim:

1. Undertake a systematic review to determine the incidence of free protein S deficiency in adult patients (without concurrent VTE) with nephrotic syndrome

2. Assess validity of laboratory assays for assessing protein S deficiency (free or total) in the included studies

Method: We searched MEDLINE and Embase databases; key search terms included "protein S" and "nephrotic syndrome", and incorporated MeSH headings. Eligibility criteria included cohort studies of adult participants with nephrotic range proteinuria (urinary protein excretion >3 grams/24 hours), with protein S quantification by any method. Articles not in English were excluded. Inclusion of studies was assessed by two reviewers (Figure 1). Data were extracted including method of protein S testing and proportion of patients with protein S deficiency (total or free), defined as levels below the lower limit of the reference interval for respective assays.

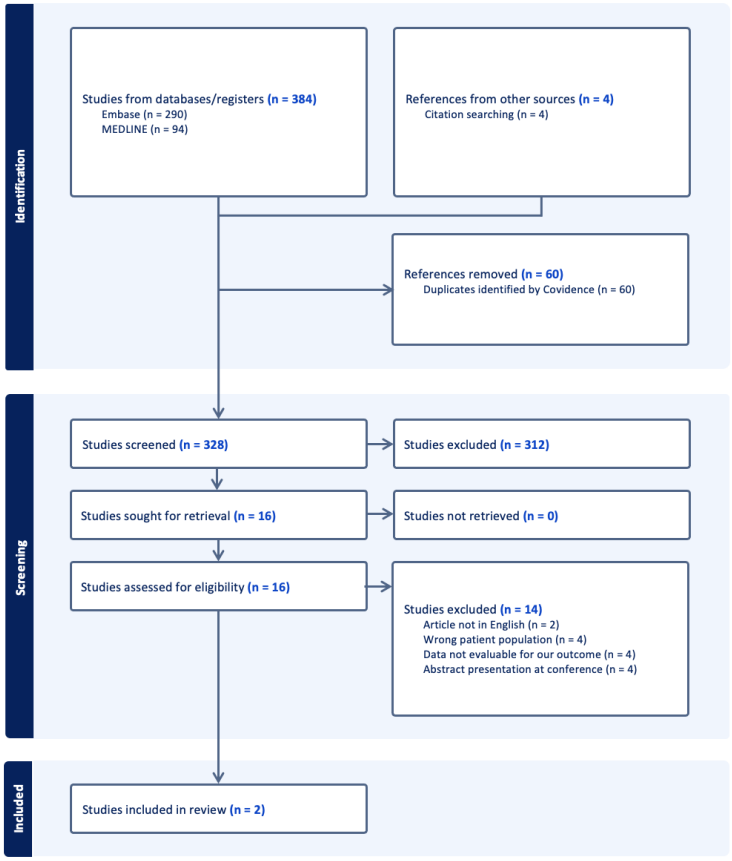


Figure 1: Studies screened and included in systematic review

Results: Two articles including a total of 39 patients were included in the final analysis. Both studies performed total protein S testing using Laurel rocket electrophoresis; neither tested free protein S. 67% of patients had a protein S level above the reference range, 26% had a protein S level in the normal range and 8%(0.7-16%) (3 patients) had a total protein S level below the lower limit of the range.

Conclusion: To date, studies have only assessed total protein S in nephrotic syndrome, which may be reduced in some patients. Free protein S, however, is more reflective of active protein S, and further studies are needed to determine if free protein S deficiency occurs in nephrotic syndrome, and if this contributes to the thrombogenicity of this condition.

References

- Kerlin BA, Ayoob R, Smoyer WE. Epidemiology and pathophysiology of nephrotic syndrome-associated thromboembolic disease. Clin J Am Soc Nephrol 2012; 7:513-520.
1. Vigano-D'Angelo S, D'Angelo A, Kaufman CE Jr, et al. Protein S deficiency occurs in the nephrotic syndrome. Ann Intern Med 1987; 107:42.
2. Gouault-Heilmann M, Gadelha-Parente T, Levent M, et al. Total and free protein S in nephrotic syndrome. Thromb Res 1988; 49:37.

Nitrous oxide abuse, hyperhomocysteinaemia and thrombosis: a case report

Murton A[1,2], Yuen H[1,2], Bennett A[1,2], Chunilal S[1,2]
[1]Monash Haematology, Monash Health, Melbourne, Australia, [2]Monash University, Melbourne, Australia

Complications of inhalational nitrous oxide (NO) are emerging in the context of rising recreational use[1]. Both venous and arterial thromboembolism have been described[2], with the proposed mechanism being marked hyperhomocysteinaemia occurring secondary to vitamin B12 inactivation by NO.

A 29-year-old female presented to her general practitioner with abdominal pain, and a diagnosis of renal vein thrombosis was made via CT abdomen; no clear provoking factors were identified at this time. She subsequently commenced apixaban 5mg twice daily, however self-ceased this shortly thereafter. Three months later, she presented to her general practitioner with pleuritic chest pain, and a CT pulmonary angiogram revealed bilateral pulmonary emboli (PE) with large thromboembolic burden. She was transferred to hospital and required emergency systemic thrombolysis due to haemodynamic instability. She was subsequently treated with enoxaparin, transitioned to apixaban therapy and discharged home. Two weeks later she represented to hospital with paraesthesias in her lower limbs with MRI spine highly suggestive for subacute combined degeneration of the spinal cord. Her total vitamin B12 level was 50pmol/L (range 140-670) and holotranscobalamin was 25.2pmol/L (range <35), consistent with vitamin B12 deficiency. Further history revealed heavy NO use of approximately 50-60kg over weekends, including in the 3-4 months preceding her thrombotic events. She has now ceased all NO use. Based on this additional information, a homocysteine level was retrospectively performed on an aliquot of her frozen plasma and was elevated at 36.3micromol/L (range 5-15).

This case describes the association between inhalational nitrous oxide abuse and venous thromboembolism, in the setting of vitamin B12 deficiency and hyperhomocysteinaemia. Recognition of this association is crucial to appropriately counsel and educate patients to reduce their risk of recurrence, and to allow for consideration of discontinuation of indefinite anticoagulation when this provoking factor is identified and eliminated, such as in our case.

References:

- Australian Institute of Health and Welfare. National drug strategy household survey 2019. https://www.aihw.gov.au/getmedia/3564474e-f7ad-461c-b918-7f8de03d1294/aihw-phe-270-NDSHS-2019.pdf.aspx; 2019 [Accessed May 2023]

1. Caris MG et al. Nitrous oxide abuse leading to extreme homocysteine levels and thrombosis in young adults: a case series. J Thromb Haemost 2023;21(2):276-283

Direct discharge of low risk pulmonary embolism patients from emergency department

Neagle J[1], Lim H[1], Chellaram V[1], Ho P[1]
[1]*Northern Health, Epping, Australia*

Aim: To assess the safety outcomes of patients with low risk pulmonary embolism (PE) directly discharged from the Emergency Department (ED) on direct oral anticoagulant (DOAC) therapy.

Method: A discharge pathway for patients presenting to the ED with low risk PE was formally implemented in July 2022. Patient data was retrieved via medical records, according to ICD-10 code for PE (I269). Only patients with a discharge diagnosis of confirmed PE and were discharged from ED or Short Stay Unit within ≤ 2 days (48 hours) were included. Data collected included basic patient characteristics, follow-up and outcomes (recurrent thrombotic event, bleeding and death).

Results: There was a total of 21 patients who were discharged via this pathway from 1 July 2022 to 31 January 2023. The median age was 54 years old (range 34 – 84 years) with 11 females (52%). The average PE severity index score (PESI) was 61 (range 34 – 94), including 15 patients with very low risk, 4 with low risk and 3 with intermediate risk PE. Inpatient Haematology consult (either via telephone or in-person) was provided for 17/21 patients and 71% (16/22) were followed up by the Anticoagulation Stewardship (ACS) Pharmacist within a week from discharge (average time 5 days). 81% of patients (17/21) were followed up in the Thrombosis Clinic with an average time of 6 weeks. Overall, there were zero instances of bleeding, clot progression or mortality within 3 months of discharge.

Conclusion: The low risk PE discharge pathway appears safe with no adverse patient outcomes, although clinician compliance with the pathway can be improved further. It appears to be a safe and practical solution to help facilitate patient flow, reduce length of stay, and improve patient experience. Ongoing safety outcomes will be monitored.

Unexpectedly Prolonged Prothrombin Time In Patient With Low Molecular Weight Heparin

Othman H[1], Kamal F[2]
[1]Hospital Al Sultan Abdullah UiTM, Bandar Puncak Alam, Malaysia, [2]Faculty of Medicine Universiti Teknologi MARA, Sungai Buloh, Malaysia

Aim: Lupus anticoagulants (LA) are non-specific plasma inhibitors that can occur spontaneously or in association with autoimmune disease such as antiphospholipid syndromes (APS). LA have been known to interfere with activated partial thromboplastin time (aPTT) test and less commonly prothrombin time (PT) test.
We reported an unusual finding of a 45-year old man who presented with abnormally prolonged PT. A subsequent prolongation of PT which did not correlate with administration of Low Molecular Weight Heparin (LMWH) triggered us to investigate.

Method: Further investigation revealed that the patient neither has bleeding tendency nor recent consumption of warfarin. A mixing test performed on Sysmex-CS2500 using Dade®Innovin® reagent revealed a Rosner Index of more than 15% indicating no correction for PT mixing. The sample was referred to another laboratory (that used different analyser and reagent) for a second opinion which however revealed a slightly prolonged PT with complete correction of PT mixing. Another repeat sample was analysed using three different reagents (two different lots of Dade®Innovin® and Thromborel®S) using Sysmex-CS2500 platform.

Results:

Equipment/ Reagent	Coagulation Testing	Result (s)	Reference range (s)
First batch of sample			
Sysmex CS-2500/ Dade® Innovin®	PT	40	9.3 -10.8
	APTT	34	22.2 – 31.6
Sysmex CA-104/ Dade® Innovin®	PT	56	
	APTT	30	
ACL Top/ HemosIL® RecombiPlasTin 2G	PT	13	
Manual method/ Dade® Innovin®	PT	53	
Second batch of sample			
Sysmex CS-2500/ Dade® Innovin® (Current lot)	PT	41	
Sysmex CS-2500/ Dade® Innovin® (Different lot)	PT	38	
Sysmex CS-2500/ Thromborel®S	PT	13	

Table 1: PT/ APTT results using different equipment and reagents

For the first batch of sample, the PT results were both prolonged when using same PT reagent (Dade®Innovin®) on Sysmex-CS2500 and Sysmex-CA104. However, PT results were normal when using ACL Top and HemosIL® (different analyser's model and reagent). For the second batch of sample, it was tested with two different lots of Dade®Innovin® and another range of CS2500's reagent, Thromborel®S using the same CS2500. Surprisingly, the PT results were normal when using Thromborel®S and remained prolonged for the Dade®Innovin®. Patient's samples were then outsourced for thrombophilia testing and result showed triple positivity for APS (positive for LA, Anti-Cardiolipin Antibodies and anti-Beta-2-Glycoprotein1).

Conclusion: These findings demonstrate that PT reagents may have different level of sensitivity towards LA and APS. Any persistent prolongation of testing which does not correlate with clinical history may warrant further investigation.

Colchicine alters ROS generation in response to GPVI stimulation in platelets

Pennings G[1], Reddel C[1], Traini M[1], Campbell H[1], Chen V[1,2], Kritharides L[1,3]

[1]ANZAC Research Institute/Sydney Local Health District, Concord, Australia, [2]Department of Haematology, Concord Repatriation General Hospital, Concord, Australia, [3]Department of Cardiology, Concord Repatriation General Hospital, Concod, Australia

Aim: Colchicine is traditionally used to treat inflammatory conditions e.g., gout, and more recently use has expanded to treat pericarditis and the prevention of heart attack and stroke. It has antiplatelet effects, the mechanisms of which are not well understood. We aimed to determine if colchicine altered platelet activation responses after stimulation of the collagen receptor glycoprotein (GP)VI and the ADP receptor $P2Y_{12}$ *in vitro*.

Method: Blood from healthy volunteers (n=4-10) was used to examine the effect of typical plasma concentrations of colchicine (20 nM) and higher, microtubule-inhibitory concentrations (2 mM). Platelet responses to stimulation of GPVI and $P2Y_{12}$ in the presence of *in vitro* colchicine (30 min, 37°C) were examined by Multiplate™ aggregometry (whole blood and platelet rich plasma [PRP]) and flow cytometry (ROS generation and platelet activation markers). Western blots (e.g., phosphotyrosine) were used to further assess the effect of colchicine on the GPVI signalling pathway. Data were analysed with students paired t-test and ANOVA with analysis for trend, $p<0.05$ was significant, presented as mean±SD.

Results: Colchicine led to a significant decrease in aggregation (whole blood and PRP, 68.1±19.2 v 60.2±16.2 AUC, p=0.006 and 78.0±10.7 v 73.3±12.9 AUC, p=0.005 respectively; vehicle v 20 nM colchicine) in response to collagen stimulation, but ADP stimulation was significant only at 2 mM colchicine. There was a significant decrease in ROS generation (H_2DCF-DA) with cross-linked collagen related peptide (CRP-XL) at both colchicine concentrations. Platelet activation marker, CD62P, was inhibited by 2 mM colchicine (both CRP-XL and ADP). Colchicine led to a significant, concentration dependent, decrease in phosphotyrosine (100 kDa) both in response to CRP-XL stimulation (trend, p=0.04) and without stimulation (trend, p=0.03).

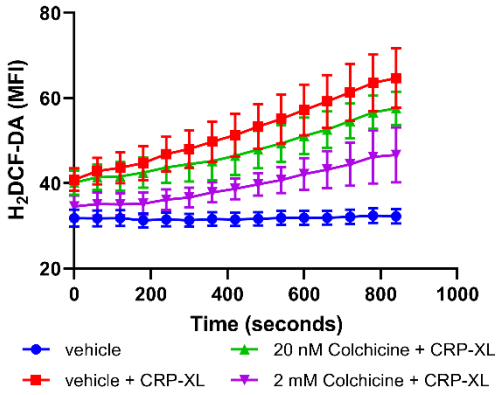


Figure: ROS generation in platelets; mean±SEM

Conclusion: Colchicine inhibits collagen-mediated platelet aggregation via the GPVI receptor at physiologically relevant concentrations, inhibits platelet degranulation at higher concentrations, and demonstrates effects on ROS and phosphotyrosine formation.

Novel Interventional Therapeutic strategy in Acquired Haemophilia A presenting with life-threatening haemorrhage

Piper K[1], Curnow J[1]
[1]*Westmead Hospital, Westmead, Australia*

Aim: We present a case of AHA in a 53yo gentleman with life-threatening haemorrhage secondary to FVIII-inhibitor development. This presentation coincided with cognitive decline that was consistent with early onset of neurodegenerative disease

Method: Case notes and investigation results were extracted from electronic medical records. Documentation and results from outpatient appointments, including follow-up appointments post discharge, were also reviewed.

Results: Our patient presented overnight to a regional hospital with severe abdominal pain, syncope, and hypotension in the setting of a large spontaneous retroperitoneal haemorrhage. He was transferred to a tertiary centre however derangements in his coagulation profile were not initially realised. Initial attempts at embolization with coils in conjunction with activation of massive transfusion protocol failed to control his life-threatening haemorrhage.
Haematology was consulted and a FVIII-inhibitor was identified (peak titre 78 Bethesda units). Novoseven® (recombinant FVIIa) infusion was commenced. A third attempt at embolization utilising the novel agent Onyx® (Ethylene vinyl alcohol copolymer), was successful in controlling bleeding and preventing impending mortality.

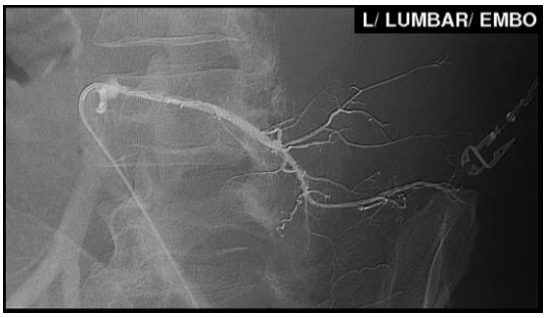


Figure 1: *Left lumbar artery following embolisation with Onyx®. Onyx® is a liquid embolization therapy that occludes blood vessels. It appears radiodense on CT scans and radiographs with a curvilinear pattern following the path of the embolized vessel.*

Novoseven® was gradually weaned in the setting of immunosuppressive therapy (Prednisone 1mg/kg daily, Cyclophosphamide 5mg/kg IV single dose and Rituxumab 375mg/m2 IV weekly for 4 doses). Spontaneous left knee haemarthrosis and initial bleeding from the femoral access site necessitated further Novoseven® therapy. Prednisone weaning was conducted in small increments with the patient remaining on 25mg oral daily until achieving normal FVIII levels with absence of inhibitor- 6 months following initial presentation.

Numerous investigations have not identified an underlying cause of the inhibitor or neurological impairment, although cognitive PET scan is pending.

Conclusion: Our case presents a novel interventional radiological strategy utilising Novoseven® and Onyx® in the management of life-threatening haemorrhage in a patient with AHA. The radiodense appearance of Onyx® in CT scans and radiographs is important to recognise.

Outcomes in Adults and Adolescents With Severe Hemophilia A in the Phase 3 XTEND-1 Study Who Switched to Efanesoctocog Alfa Prophylaxis from an Observational Study With Factor VIII Prophylaxis

P'Ng S[1], Susen S[2], Kulkarni R[3], Nogami K[4], Peyvandi F[5,6], Konkle B[7], Santagostino E[8], Khan U[9], Willemze A[10], Bystricka L[8], Dumont J[11], Chowdary P[12]
[1]Haemophilia And Haemostasis Centre, Fiona Stanley Hospital, Perth, Australia, [2]Centre Hospitalier Universitaire de Lille, Université de Lille, Lille, France, [3]Department of Pediatrics and Human Development, Michigan State University, East Lansing, USA, [4]Nara Medical University, Nara, Japan, [5]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [6]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy, [7]Washington Center for Bleeding Disorders and the University of Washington, Seattle, USA, [8]Sobi, Stockholm, Sweden, [9]Sanofi, San Diego, USA, [10]Sanofi, Amsterdam, Netherlands, [11]Sanofi, Cambridge, USA, [12]Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital, London, UK

Aim: Efanesoctocog alfa (50 IU/kg), a new class of factor VIII (FVIII) replacement therapy designed to decouple recombinant FVIII (rFVIII) from endogenous von Willebrand factor, provides high sustained FVIII activity in the normal to near-normal range (>40 IU/dL) for the majority of the week with once-weekly dosing. Here, we describe a post-hoc intrapatient comparison of outcomes in patients (≥12 years old) with severe hemophilia A on conventional standard-of-care (SOC) FVIII prophylaxis in a prospective, observational study prior to switching to efanesoctocog alfa prophylaxis while enrolled in XTEND-1 (NCT04161495).

Methods: After providing informed consent, 78 patients (12–69 years old) received ≥6 months standard-of-care FVIII prophylaxis (standard half-life FVIII [recombinant or plasma-derived FVIII; n=44] or extended half-life rFVIII [n=34]) in the observational study, and then enrolled in Arm A of XTEND-1 (52 weeks, once-weekly efanesoctocog alfa [50 IU/kg]). Annualized bleed rates (ABR), treatment of bleeds, FVIII consumption, and injection frequency were compared for conventional SOC FVIII prophylaxis (pre-study) versus efanesoctocog alfa (on-study).

Results: Mean (95% confidence interval [CI]) change in overall ABR from pre-study to on-study was −2.27 (−3.44, −1.10); change in joint, spontaneous, and spontaneous joint ABRs were −1.55 (−2.46, −0.64), −1.20 (–1.84, −0.57), and −0.93 (−1.42, −0.43), respectively. Overall, 42.3% of patients were bleed-free pre-study (median observation period: 50.05 weeks) versus 64.1% on-study (median: 50.09 weeks). Mean (standard deviation) number of injections for bleed treatment was 1.8 (5.10) pre-study versus 1.1 (0.30) on-study. Mean (95% CI) change in weekly consumption was −35.00 (−47.82, –22.18) IU/kg and change in annualized consumption was −1828.22 (−2491.88, −1164.57) IU/kg. Mean (95% CI) change in annual injection frequency was −71.7 (−83.2, −60.2).

Conclusion: Switching to once-weekly efanesoctocog alfa 50 IU/kg prophylaxis provided superior bleed protection with reduced injection frequency and lower weekly FVIII consumption versus prior SOC FVIII prophylaxis.
Word count: 296/300

Feasibility of real-time in-vivo assessment of peripheral intravenous catheter micro-motion using two- and three-dimensional ultrasound in human participants

Pritchard R[1], Clarke A[1], Snelling P[2,3], Fontanarosa D[4], Fozzard N[1], Woodroffe A[1], Vayani Z[1], Bulmer A[1]

[1]Alliance for Vascular Access Teaching and Research Group (AVATAR), Experimental Laboratory Science (XlabS) Research Group and the School of Pharmacy and Medical Sciences, Griffith University, Southport, Australia, [2]Emergency Department, Gold Coast University Hospital, Southport, Australia, [3]School of Medicine and Dentistry, Griffith University, Southport, Australia, [4]School of Clinical Sciences, Queensland University of Technology, Brisbane, Australia

Aim: Catheter micromotion is a commonly used yet poorly defined term. With catheters predominantly sitting against the surface of veins, minute movement is assumed to abrade the intimal surface, contributing to device failure. We have utilised a combination of ultrasound imaging combined with motion tracking software and machine learning to define and quantify catheter micro-motion during clinician interaction.

Method: A cohort of 5 participants were cannulated bilaterally in their forearms with two different catheter designs. Participants were repeatedly monitored at AM and PM assessments for a period of 72 hours or until device failure due to loss of patency. At each timepoint, two- and three-dimensional ultrasound images of catheter tips, vessel segments and echogenic (presumed thrombotic) material were collected and assessed to document catheter micro-motion during clinician interaction (i.e. during routine removal of extension set from skin, attachment of flushing syringe and unclamping extension set).

Results: Two-dimensional assessment of catheter micro-motion within the lower arm cephalic vein (in transverse plane) during clinician was demonstrated to be feasible, taking no longer than 25 seconds and was performed in all participants, bilaterally. Catheter tip displacement in the x, y plane and total distance travelled was routinely observed and quantified and data transferred to REDCAP database (statistical analysis cannot be provided until database lock), every 125 ms (3 Hz). Rendering vessel segments in three-dimensions allowed for the location of the catheter tip in x, y and z planes to be defined in relation to a static anatomical landmark to determine motion of the device pre- and post-assessment.

Conclusion: Documented here is a method developed to accurately measure aspects of catheter micro-motion within human participants. The combination of multiple ultrasound imaging modalities, coupled with advancements in machine learning and motion tracking software provide the ability to track catheter motion non-invasively and over several clinical visits.

Haemostasis/thrombosis genetics multidisciplinary meeting development: a single-centre experience.

Ramanan R[1,2], McFadyen J[1,2], Tran H[1,2], Perkins A[1,2]
[1]Alfred Health, Melbourne, Australia, [2]Monash University, Melbourne, Australia

Aim: To describe the experience of a multidisciplinary meeting (MDM) created at Alfred Health to discuss complex cases arising from performance of a haemostasis thrombosis genetics panel via whole exome sequencing (WES).

Method: Genetic testing performed via WES with analysis targeted to a defined set of genes associated with bleeding, platelet and thrombotic disorders. Genes chosen by an expert panel of clinicians, pathologists and scientists after review of the International Society on Thrombosis and Haemostasis (ISTH) Tier 1 Gene List and published literature. Variant curation performed in reference to the American College of Medical Genetics and Genomics (ACMG) guidelines with specifications applied if published by the ClinGen working group. Discussion of complex cases held at a monthly MDM initiated in Dec 2022 attended by reporting pathologists, scientists, treating clinicians and genetic counsellors.

Results: Indications for genetic testing include patients with bleeding or thrombotic disorders of unknown cause, those with a clinically confirmed disorder requiring genetic confirmation and screening of family members.

Discussion at MDM includes clinical significance of variants identified including management strategies, discussion of possible means of upgrading/downgrading variants of uncertain significance (VUS), need to screen proband for non-haemostatic clinical features associated with variants (including incidental findings), need for familial screening and need for genetic counselling.

One variant of interest identified to date is a suspected *F7* deletion associated with two pathogenic/likely pathogenic variants on the alternate allele each at 100% VAF in a patient with FVII deficiency. Another is a novel synonymous *FGG* variant with predicted aberrant splicing associated with hypofibrinogenemia, which was initially not detected as synonymous variants were filtered out by the bioinformatics pipeline.

Conclusion: Development of an MDM in the haemostasis and thrombosis genetics space has resulted in a useful forum to discuss novel variants and the complexities associated with genetic discovery.

Age-related changes in overall haemostatic potential

Reddel C[1], Pennings G[1], Chen V[1,2], Gnanenthiran S[1,3], Kritharides L[1,3]
[1]*ANZAC Research Institute, Concord Repatriation General Hospital, Concord, Australia,*
[2]*Department of Haematology, Concord Repatriation General Hospital, Concord, Australia,*
[3]*Department of Cardiology, Concord Repatriation General Hospital, Concord, Australia*

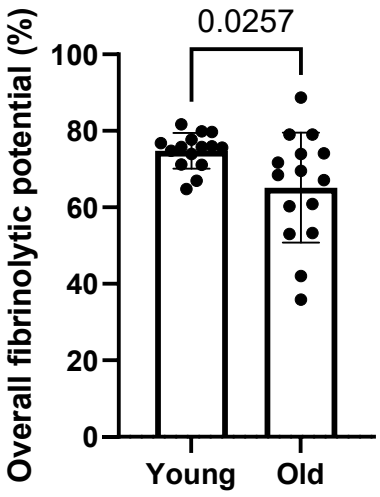
Aim: The haemostatic system becomes unbalanced with ageing, with increased incidence of both thrombotic events and bleeding. Laboratory assays suggest a hypercoagulable and hypofibrinolytic profile. Extracellular vesicles (membrane-bound extracellular particles released by cells) can support coagulation. We aimed to identify dynamic changes in coagulation with healthy ageing and their links to cell-specific extracellular vesicles.

Method: Citrated platelet-free plasma was collected and stored from healthy older (age ≥70 y, n=15) and young (20-30 y, n=15) community-dwelling people. We tested dynamic coagulation and fibrinolysis using the Overall Haemostatic Potential (OHP) assay, generating fibrin time curves by turbidometry with added tissue factor and tissue plasminogen activator. We measured circulating extracellular particles (EP) stained for phosphatidylserine and platelet, endothelial and leukocyte markers using flow cytometry. Between-group comparisons were made using Mann-Whitney U tests and associations between markers identified using Spearman correlations. Results are presented as median [confidence interval].

Results: The OHP assay identified a hypercoagulable and hypofibrinolytic state in healthy elderly people, with faster and more extensive fibrin generation and slower clot lysis, and with higher variation in the older cohort (e.g. overall fibrinolytic potential 76 [71-78]% young, 68 [53-74]% elderly, p=0.026, Figure 1). The assay also demonstrated an increased delay to fibrin clot formation (14.1 [10.6-17] min young, 16.4 [15.6-19.2] min elderly, p=0.049). While several EP populations correlated with OHP parameters, there were no differences between old and young cohorts in any EP population.

Conclusion: The OHP assay confirms a hypercoagulable and hypofibrinolytic profile in healthy elderly people, with no age-related link to cell-specific extracellular particle/vesicle populations. A paradoxically lengthened delay to clot formation could predispose to bleeding. These findings support the importance of developing age-specific normal ranges for coagulation studies and future studies testing links between OHP parameters and clinical risk factors.

Figure 1. Overall fibrinolytic potential results.



Fibrinogen Austin (Aa17Gly→Cys and Aa381Ser→Phe); Identification of a novel fibrinogen mutation causing dysfibrinogenaemia.

Rigano J[1], Lee N[1]
[1]*Northern Health, Epping, Australia*

Background: Fibrinogen is encoded by *FGA*, *FGB* and *FGG* genes on chromosome 4q. The 340 kDa glycoprotein is organised as two identical heterotrimers consisting of A□, Bβ and γ chains. Mutations causing hypofibrinogenaemia and/or dysfibrinogenaemia phenotypically give rise to haemorrhage and/or thrombosis. This report describes two novel fibrinogen mutations in a woman causing dysfibrinogenaemia with a bleeding phenotype.

Methods: The proband a 51-year-old female of Chinese ancestry presented for investigation of menorrhagia. She had a history of mild bleeding from uterine fibroids, a haemorrhoidectomy and following three vaginal births. Ten other family members were also investigated. Coagulation assays were performed on the ACL TOP CTS 500 analyser. Fibrinopeptide release assays, HPLC fibrinogen, SDS-PAGE, whole protein time-of-flight mass spectrometry (TOF MS) and DNA sequencing were performed to assess the physical, functional and molecular properties of fibrinogen.

Results: Coagulation investigations and DNA analysis of the proband and family members are shown in Table 1. Fibrinopeptide release assays showed delayed polymer formation and 50% decrease release of fibrinopeptide A. Following addition of thrombin, the proband's fibrin clots remained sloppy after 20 minutes. Released peptides were quantitated resulting in a low A:B ratio of 0.53. SDS-PAGE, Whole protein TOF MS revealed abnormal masses of A□ chains. DNA sequencing showed the proband was heterozygous for three sequence changes in the A□ chain; A□312Thr→Ala known benign polymorphism and two novel mutations A□17Gly→Cys and A□381Ser→Phe. Thrombin cleaves the A□Arg^{16}-Gly^{17} bond releasing Fibrinopeptide A as the initial step in fibrin polymerisation implicating the A□17Gly→Cys as the cause of dysfibrinogenaemia. The proband showed delayed polymer formation implicating A□381Ser→Phe which occurs in the connector region of the A□C domain. This novel double mutation variant was named fibrinogen Austin.

Conclusions: Here reported was the identification of two novel fibrinogen mutations in a woman causing dysfibrinogenaemia. Investigation of extended family members revealed the mutations were co-inherited on the same *FGA* allele.

	COAGULATION ASSAYS						*FGA* MUTATIONS		
	PT	APTT	FIB-D	FIB-C	TT	RT	17G→C	381S→F	312T→A
PROBAND	13	26	1.9	0.5	45	52	GC	SF	TA
MOTHER	11	25	2.8	3.0	14	17	GG	SS	TA
FATHER	15	30	1.6	0.3	39	57	GC	SF	TT
SON	14	31	1.7	0.2	41	59	GC	SF	TT
SON	12	29	2.5	2.6	16	19	GG	SS	TA
SON	12	30	2.7	2.9	15	18	GG	SS	TA
SISTER	14	26	1.5	0.3	33	56	GC	SF	TA
BROTHER	15	28	1.5	0.3	38	54	GC	SF	TT
BROTHER	15	33	1.4	0.3	36	55	GC	SF	TT
BROTHER	13	27	1.9	2.5	17	20	GG	SS	TT
BROTHER	15	32	1.9	0.3	42	56	GC	SF	TA
NORMAL	11-15 s	22-38 s	2.0-4.0 g/L	2.0-4.0 g/L	11-17 s	15-22 s	GG	SS	TT

Table 1. Coagulation assay and DNA analysis results of the proband and ten family members.

Calibration of WHO 3rd International Standard for von Willebrand Factor Concentrate, value assignment of WHO 2nd International Standard for Factor V and Calibration of SSC/ISTH Secondary Coagulation Standard Lot #5.

Rigano J[1]
[1]***Northern Health, Epping, Australia***

Introduction: The Scientific and Standardisation Committee (SSC) of the ISTH develops standards which are adopted by the WHO as International Standards. Diagnostic Manufacturers use the standards for labelling of coagulation calibrators and controls. The National Institute for Biological Standards and Controls (NIBSC) internationally facilitates the preparation, evaluation and distribution of International Biological Standards. The aim of this study was the collaborative participation in the value assignment of the WHO 2nd International Standard (IS) for FV, calibration of the SSC/ISTH Secondary Coagulation Standard (SCS) Lot #5 and calibration of the WHO 3rd IS for vWF Concentrate.

Methods: For the WHO 3rd IS, four sets of five samples were used, assayed in duplicate at three different dilutions. A total of 120 vWF Antigen (vWF:Ag) and vWF Ristocetin Cofactor (vWF:RCo) assays were performed. For the WHO 2nd IS, four sets of five plasmas were used, assayed in duplicate at four different dilutions. A total of 160 FV assays were performed. For the SSC/ISTH SCS Lot #5, four sets of two plasmas were used, assayed in triplicate at four different dilutions. A total of 96 FVIII and FIX assays were performed. Comparisons between test data were made by two-tailed t-test of log transformed laboratory mean estimates. Variability within and between laboratories were expressed using geometric coefficients of variation (GCV).

Results: Values assigned to WHO 3rd IS for vWF Concentrate was *12.0 IU/ampoule* for vWF:Ag and *8.7 IU/ampoule* for vWF:RCo. Value assigned to WHO 2nd IS for FV was *0.72 IU/mL.* Values assigned to SSC/ISTH SCS Lot #5 for FV was *0.87 IU/mL*, for FVIII was *0.82 IU/mL* and for FIX was *1.09 IU/mL*. Overall, there was low inter-laboratory variability and very good agreement between the mean laboratory estimates. This collaboration involved 22 countries and 48 participating laboratories.

Conclusions: The NIBSC successfully facilitated the collaborative study to value assign WHO 3rd IS for vWF Concentrate, WHO 2nd IS for FV and SSC/ISTH Secondary Coagulation Standard Lot #5 for FV, FVIII and FIX.

Familial multiple coagulation factor deficiencies (FMCFDs); A rare case of combined deficiency of factor V and factor VIII (F5F8D)

Rigano J[1]
[1]*Northern Health, Epping, Australia*

Introduction: FMCFDs are characterised by the presence of more than one coagulation factor deficiency arising from a genetic defect or defects. The three subgroups of disorders include FMCFDs arising from: (i) co-inherited single coagulation factor deficiencies (ii) a single genetic defect (iii) cytogenetic abnormalities. F5F8D is caused by mutations in either the *LMAN1* or *MCFD2* genes responsible for the transportation of FV and FVIII from the ER to the Golgi for post-translational modification and secretion into the circulation. This case describes a child with F5F8D that was not initially diagnosed at presentation of bleeding.

Methods: A 3-year-old girl requiring treatment for a post-operative infection presented with intermittent bleeding. She had recently required sutures for a lip bleed caused by a fall and previously had a lip injury which bled for three days. Initial PT and APTT were markedly prolonged and post-operative haemoglobin was 79 g/L. was administered with a slight improvement in APTT only. She received IV vitamin K and Prothrombinex® with a slight improvement in PT and normalised APTT. NovoSeven® was given to cease bleeding prior to discharge. Two weeks later PT and APTT were repeated and consistently markedly prolonged. Factor assays were then requested.

Results: Initial PT and APTT suggested common pathway factor deficiency hence vitamin K administration. PT and APTT mixing studies corrected immediately and after incubation excluding an inhibitor. Factor assays revealed deficient levels of FV and FVIII. (Table 1). Repeat analysis confirmed FV and FVIII deficiencies. F5F8D is a rare autosomal recessive congenital bleeding disorder common in consanguineous families from middle eastern countries. Patients present with prolonged bleeding following trauma or surgery. Bleeding episodes are treated on demand with DDAVP, FVIII concentrates and FFP.

Conclusions: Here reported was the detection of F5F8D in a child who presented with bleeding following trauma and prolonged PT and APTT.

ASSAY	ON ADMISSION	10 mg IV VITAMIN K	30 IU/kg PROTHROMBINEX	2 WEEK FOLLOW UP	REFERENCE RANGE
PT	45	42	33	43	11-15 s
APTT	105	78	28	100	22-38 s
PT MIX					
IMMEDIATE				12	11-15 s
2 HRS @ 37°C				13	11-15 s
APTT MIX					
IMMEDIATE				34	22-38 s
2 HRS @ 37°C				37	22-38 s
FACTOR II				82	50-120 %
FACTOR V				2	50-120 %
FACTOR VII				102	50-120 %
FACTOR X				105	50-120 %
FACTOR VIII				23	50-200 %
FACTOR IX				74	50-140 %
FACTOR XI				85	50-140 %
FACTOR XII				107	50-140 %
vWF:Ag				91	50-200 %
vWF:RCF				87	50-200 %

Table 1. Coagulation investigation results.

Discrepancy between genotype and phenotype for factor V Leiden mutation in recipients of liver and stem cell transplantation.

Rigano J[1]

[1]*Northern Health, Epping, Australia*

Background: Activated protein C resistance (APCR) was first described in 1993 (Dahlbäck *et al.*) and is the most common hereditary risk factor for venous thromboembolism (VTE) in Caucasians. In approximately 90–95% of cases, the coagulation disorder results from the factor V Leiden (FVL) mutation causing activated FV to be resistant APC cleavage resulting in a hypercoagulable state. This report describes 2 cases; one acquired APCR without FVL mutation following liver transplantation (LTX) and the other inherited APCR without FVL mutation following stem cell transplantation (SCTX) both associated with deep vein thrombosis (DVT).

Methods: The first case was a 68-year-old male presenting with DVT nine months post LTX. The second case was a 42-year-old female who presented with a CRT four months post SCTX for AML. Both patients reported no history of thrombosis prior to transplantation. Thrombophilia assays (protein C, protein S, antithrombin, APCR, lupus anti-coagulant, anti-cardiolipin and anti-β_2-glycoprotein I antibodies) were performed on the ACL TOP CTS 500. Molecular thrombophilia assays for FVL and prothrombin gene (G20210A) mutations were performed using the Qiagen Rotor-Gene by PCR and HRM analysis.

Results: For both patients, thrombophilia assay results are shown in Table 1. In case one, APCR was detected in the donor liver and the recipient's peripheral leucocyte DNA lacked the FVL mutation. In case two, APCR was detected in the recipient's liver and the peripheral leucocyte DNA of the donor lacked the FVL mutation. Since both patients reported no history of thrombosis, thrombophilia testing was never previously indicated. It has been established that LTX and SCTX recipients are at risk of VTE. Therefore, consideration should be given to thrombophilia testing of LTX and SCTX donors and recipients which may indicate anticoagulation to prevent additional morbidity.

Conclusions: Here reported were two cases of genotype and phenotype discrepancies for FVL mutation in recipients of LTX and SCTX both associated with DVT.

	THROMBOPHILIA ASSAYS										
	PC	PS	AT	APCR	APTT	DRVV S	DRVV C	aCL	aβ2Gpl	FVL GENE	PT GENE
CASE 1	102	138	113	1.75	35	1.07	1.03	2	1	NOT DETECTED	NOT DETECTED
CASE 2	127	99	105	1.64	32	1.11	1.06	7	4	NOT DETECTED	NOT DETECTED
NORMAL	70–140 %	66–154 %	80–120 %	2.2–3.3	22–38 s	<1.2	<1.2	0–20 U/mL	0–15 U/mL	NOT DETECTED	NOT DETECTED

Table 1. Thrombophilia assay and DNA analysis results.

Catheter-Related Arterial Thrombosis in a Single Paediatric Intensive Care Unit

Runge A[1,2], Roy J[2,3]
[1]*Queensland Children's Hospital, Brisbane, Australia,* [2]*Pathology Queensland, Brisbane, Australia,* [3]*University of Queensland, Brisbane, Australia*

Aim: To determine the incidence, diagnostic and management practices, short-term outcomes, and complications of symptomatic catheter-related arterial thrombosis (CAT) in patients admitted to a single paediatric intensive care unit (PICU) to assess the standard of current care and inform the creation of an institutional guideline for the management of CAT.

Method: A database search was performed for all patients admitted to a single PICU and diagnosed with CAT from November 2014 to December 2019. 56 CAT events were identified. A clinical audit was completed with manual review of relevant medical records. Descriptive data is presented.

Results: 47(83.9%) of CAT events occurred in infants (less than 12 months of age). 35(62.5%) events were indwelling arterial catheter related arterial thromboses (IC-CAT) while 21 (37.5%) were cardiac catheter related arterial thromboses (CC-CAT). 55 (98.2%) events were diagnosed by doppler ultrasonography. Unfractionated heparin was the most common therapy used (88%). Systemic thrombolysis was more frequently used for CC-CAT than for IC-CAT (71.4% vs 14.3%). Total resolution of thrombus by 14 days was demonstrated in 20 (35.7%). The most common complication of CAT was skin necrosis requiring wound debridement, occurring in 8 (14.3%). Limb amputation was required in 4 (7.1%). There were no deaths directly attributable to CAT, however one patient died during the 14-day follow-up period.

Conclusion: This audit demonstrated a delay in referral to appropriate specialists with expertise in the management of CAT, with wide variability in the documentation of clinical findings at diagnosis and follow-up, use, and timing of imaging to assess for CAT progression. There was also a significant difference in choice of treatment modality between CC-CAT and IC-CAT.
Given the demonstrated variability in diagnostic and management practices, an institutional guideline has been developed to standardise the management of CAT.

Venous thromboembolism prevention practices: a review of patients with hospital acquired venous thromboembolism

Slocombe A[1], Goulopoulos A[1], Wembridge P[1]
[1]Eastern Health, Box Hill, Australia, [2]Royal Melbourne Hospital, Melbourne, Australia

Aims: To describe patients with hospital acquired venous thromboembolism (VTE) and assess rate and accuracy of VTE risk assessment and associated prevention strategies at a metropolitan health service.

Method: All adult patients (>18 years) admitted to a metropolitan health service from 1st January to 31st December 2022 with a diagnosis of hospital acquired VTE by International Classification of Diseases codes (ICD-10) were identified from hospital records. Electronic medical records were used to obtain patient demographics, medication and clinical history.

Results: A total of 434 patient encounters were coded as having developed a VTE. Patients with repeat presentations, incorrect diagnosis or admission dates were excluded from subsequent analysis. A cohort of 52 patients remained (median age 70 years (range 19 – 96); 58% female). The median time to VTE diagnosis from admission was 15.5 days. VTE risk assessments were not performed on 12 (23%) patients, and of the 40 who had assessments 5 (12.5%) were incorrect.

VTE prophylaxis was prescribed for 44 (85%) patients. Of these, 8 (15%) were not prescribed any VTE prophylaxis despite only two having contraindication to prophylaxis. Of the patients who received prophylaxis, 5 (11%) were prescribed an inappropriate dose. The median time to initiation of VTE prophylaxis was 28.2 hours (range -0.02 to 7.2 days). An assessment of overall VTE prophylaxis management appropriateness found suboptimal management in 13 (25%) patients. Omitted or incorrect VTE risk assessment was associated with suboptimal VTE prophylaxis compared to patients with correctly completed risk assessments (41% vs 17% p=0.054).

Conclusion: This review demonstrates that 25% of patients with hospital acquired VTE were prescribed suboptimal VTE prophylaxis and subsequently developed potentially avoidable complications. This highlights that targeted strategies to optimise compliance with current VTE guidelines are needed to improve patient outcomes.

Venous anomalies – an under-recognised but important cause of venous thromboembolism (VTE)

So J[1], Dix C[1], Clements W[2,3,4], Gibbs H[5], Tran H[1,6], McFadyen J[1,6,7,8]
[1]Department of Clinical Haematology, Alfred Health, Melbourne, Australia, [2]Department of Radiology, Alfred Health, Melbourne, Australia, [3]Department of Surgery, Central Clinical School, Monash University, Melbourne, Australia, [4]National Trauma Research Institute, Melbourne, Australia, [5]Department of General Medicine, Alfred Health, Melbourne, Australia, [6]Australian Centre for Blood Diseases, Central Clinical School, Monash University, Melbourne, Australia, [7]Atherothrombosis and Vascular Biology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia, [8]Baker Department of Cardiometabolic Health, University of Melbourne, Melbourne, Australia

Aim: Venous anatomical variants, such as May-Thurner syndrome (MTS) and inferior vena cava (IVC) variants, increase risk of VTE but are under-recognised despite affecting management options including endovascular intervention. We aimed to identify the incidence of anatomical variants in deep vein thrombosis (DVT) cases involving the iliofemoral, bilateral lower limb veins or IVC.

Method: DVT cases were identified retrospectively from Alfred Health medical records using discharge ICD-10 codes between January 2014 and December 2021. Proximal DVT (excluding popliteal vein), bilateral lower limb DVT or IVC thrombus cases were included based on radiology findings (Figure 1). Demographic and clinical data were also obtained. Ethics approval was granted by our institutions Ethics Committee (approval 460/22).

Results: Over an eight-year period, there were 5731 DVT cases, with 1174 meeting inclusion criteria diagnosed by ultrasound (95%), CT scan (3%), or formal angiography (1%). The median age was 63 years, and 730 (62%) were male. 774 (63%) were provoked and 508 (43%) had risk factors such as malignancy (16%) or previous VTE (20%). 36 cases of anatomical variants (3.1%) were identified; 25 MTS and eleven hypoplastic IVC variants. Compared to all DVT cases, they were more frequently unprovoked (81% vs 37%), younger (median age 37 vs 63 years), female (67% vs 38%) and had a significant family history (11% vs 5%) (Figure 2). MTS/IVC variants were more likely to receive interventional management, such as catheter directed thrombolysis (58% vs 3%) or angioplasty (47% vs 2%), and commence indefinite anticoagulation (83% vs 33%).

Conclusion: We found 3.1% of all bilateral/proximal DVTs or IVC thromboses had an anatomic variant, but in females under 50 years, it was 15.6%. These cases often required interventional management and long-term anticoagulation to prevent recurrence. Our results suggest that further investigation for anatomical variants should be considered in these high-risk patients as this significantly impacts management.

Figure 1: Study population

Figure 2: Graph of risk factors for anatomical variants in comparison to all venous thromboembolism (VTE) cases identified

Bleeding, FVIII activity, and safety 3 years after gene transfer with valoctocogene roxaparvovec: Results from GENEr8-1

Tan C[1], Mahlangu J[2], von Drygalski A[3], Shapiro S[4], Chou S[5], Ozelo M[6], Kenet G[7], Peyvandi F[8], Madan B[9], Laffan M[10], Dunn A[11], Mason J[12], Quon D[13], Leavitt A[14], Oldenburg J[15], Chambost H[16], Reding M[17], Jayaram K[18], Yu H[18], Robinson T[18], Pipe S[19]

[1]Royal Adelaide Hospital, Adelaide, Australia, [2]Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand and NHLS, Johannesburg, South Africa, [3]Department of Medicine, University of California San Diego, San Diego, USA, [4]Oxford University Hospitals National Health Service Foundation Trust, Oxford, UK, [5]Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [6]Hemocentro UNICAMP, Department of Internal Medicine, School of Medical Sciences, University of Campinas, Campinas, Brazil, [7]The National Hemophilia Center, and Amalia Biron Research Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer, Tel Aviv University, , Israel, [8]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy, [9]Guy's & St. Thomas' NHS Foundation Trust, London, UK, [10]Centre for Haematology, Imperial College London, London, UK, [11]Nationwide Children's Hospital Division of Haematology, Oncology and Bone Marrow Transplant and The Ohio State University College of Medicine, Columbus, USA, [12]Queensland Haemophilia Centre, Cancer Care Services, Royal Brisbane and Women's Hospital, Brisbane, Australia, [13]Orthopaedic Hemophilia Treatment Center, Los Angeles, USA, [14]Adult Hemophilia Treatment Center, University of California San Francisco, San Francisco, USA, [15]Institute of Experimental Haematology and Transfusion Medicine and Center for Rare Diseases, University Hospital Bonn, Bonn, Germany, [16]AP-HM, Department of Pediatric Hematology Oncology, Children Hospital La Timone, Aix Marseille University, INSERM, INRA, C2VN, Marseille, France, [17]Center for Bleeding and Clotting Disorders, University of Minnesota, Minneapolis, USA, [18]BioMarin Pharmaceutical Inc, Novato, USA, [19]Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, USA

Aim: Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) provides endogenous factor VIII (FVIII) production to prevent bleeding in people with severe hemophilia A. The aim is to evaluate outcomes 3 years after receiving valoctocogene roxaparvovec.

Method: The open-label, multicenter phase 3 GENEr8-1 trial (NCT03370913) evaluated 6x1013 vg/kg valoctocogene roxaparvovec in 134 adult men with severe hemophilia A (FVIII ≤1 IU/dL) without inhibitors. Bleeds and FVIII use were self-reported after regular prophylaxis ended (scheduled for week [W] 4) through data cutoff. Comparisons to baseline on FVIII prophylaxis were performed in 112 HIV-negative participants enrolling from a non-interventional study (rollover population). FVIII activity per chromogenic assay and quality of life (QOL) per Haemo-QOL-A were assessed in 132 HIV-negative participants (modified intent-to-treat [mITT] population). Safety was assessed in all participants.

Results: Median follow-up was 162 weeks (N=134); 131 participants completed W156. Over 3 years in 112 rollover participants, mean annualized treated bleeding rate was 0.8 bleeds/year, mean annualized rate of all bleeds was 1.3 bleeds/year, and mean FVIII utilization was 125 IU/kg/year. During year 3, 73.2% of 110 rollover participants had zero treated bleeds and 61.6% had no bleeds (excluding surgeries/procedures). At W156, mean and median FVIII were 18.8 and 8.4 IU/dL (mITT, N=132); 11.4%, 56.1%, and 32.6% of mITT participants had FVIII activity ≥40 IU/dL, ≥5 and <40 IU/dL, and <5 IU/dL, respectively. Overall, 10/132 (7.6%) participants resumed prophylaxis. Mean Haemo-QOL-A Total Score improvement from baseline to W156 was 6.6 (n=122; P <0.0001), exceeding the anchor-based clinically important difference (5.5). No new safety signals emerged. Since the previous data cut, 34/134 participants (25.4%) had alanine aminotransferase (ALT) elevation, mostly Grade 1, but none initiated immunosuppressants. Overall, 106/134 (79.1%) used corticosteroids for ALT elevation for median 33 weeks.

Conclusion: Valoctocogene roxaparvovec provided robust hemostatic efficacy relative to FVIII prophylaxis for 3 years, with QOL improvement and stable safety.

Demographics and outcome of patients with congenital haemophilia in Sarawak, Malaysia

Tang A[1], Leong T[1], Yong K[2], Lee G[1], Ko C[1], Chew L[1]
[1]Haematology Unit, Department of Internal Medicine, Sarawak General Hospital, Kuching, Malaysia, [2]Department of Internal Medicine, Miri Hospital , Miri , Malaysia

Aim: Sarawak, the largest state in Malaysia, has geographically and characteristically widely varied population. We aimed to study the demographics and outcome of patients with congenital haemophilia in Sarawak.

Method: A cross-sectional study was conducted in 2023 at four haemophilia treatment centres in Sarawak. Demographics and clinical data were compiled in Sarawak Haemophilia Registry.

Results: 115 patients with congenital bleeding disorders were identified - 79(68.7%) haemophilia A(HA) and 21(18.3%) haemophila B(HB). The others being non-haemophilia bleeding disorders, totaling 15 patients were Von-willebrand disease (4), FVII (3), FX (5) and FXI (3) deficiency. All except one were males. Severe haemophilia patients were noted in 53.2%(42/79) of HA and 61.9%(13/21) of HB. Approximately half of HA(48.1%) and HB(52.4%) population had no identifiable family history of haemophilia. About two-thirds of severe HA were on prophylaxis [27/42(64.3%)] and one-third [4/13(30.8%)] in severe HB. Among those on prophylaxis, three severe HA patients with inhibitors received emicizumab with remarkable reduction in bleeding events; one female moderate HA patient on PEGylated recombinant anti-haemophilic factor; whereas others [27/31(87.1%)] on plasma-derived factor concentrate. Immune tolerance induction was initiated in three HA paediatric patients using a dose regimen of 100IU/kg/day with only one success. Inhibitors developed in 9/79(11.4%) of the HA population [3/79(3.8%) high responders]. The median inhibitor titre was not significantly different between on-demand and prophylaxis groups (1.0BU versus 2.0BU; *p*-value 0.297, Mann-Whitney test). None of the patients developed inhibitory alloantibodies to factor IX. Eleven (11.6%) underwent radiosynovectomy. Three patients succumbed - two attributed to intracranial bleed; one gastrointestinal bleed. The overall incidence of HA and HB was 1 in 12,664 and 1 in 40,104, respectively.

Conclusion: Our incidence of congenital haemophilia is considerably lower than reported data. This study outlines haemophilia landscape in Sarawak and offers objective standards for forward planning.

Effect of emicizumab prophylaxis on analgesic requirements in patients with haemophilic arthropathy

Wan C[2], Powell A[1,2], Cheng J[1,2], Tran H[1], McFadyen J[1], Polus A[1]
[1]Alfred Hospital, Melbourne, Australia , [2]Department of Medicine, Central Clinical School, Monash University, Melbourne , Australia

Aim: Joint disease is a major cause of morbidity in patients with haemophilia. Emicizumab has been shown to be effective in reducing bleeding rates and improving joint outcomes in patients with haemophilia A (PWHA). However, the impact of emicizumab on analgesic requirements has not been previously reported. This study aimed to explore the analgesic requirements and joint outcomes in PWHA before and after the use of emicizumab.

Method: Data was collected by a retrospective chart review of the electronic medical records of PWHA with joint disease who received ≥ 6 months of emicizumab prophylaxis between 01 January 2018 to 01 May 2023 in a state-wide haemophilia referral centre. Use of pharmacological and non-pharmacological analgesia was collected at baseline and after ≥ 6 months of emicizumab.

Results: 51 eligible patients who had received emicizumab prophylaxis were identified, of which 39 had their analgesic regimen recorded as per the inclusion criteria. Pharmacological analgesia was used by 33.3% of patients prior to commencement of emicizumab. Use of paracetamol, NSAID and opioid-class medication were used in 18.2%, 15.2% and 15.2% respectively. After ≥ 6 months of emicizumab prophylaxis, 30.3% of patients used pharmacological analgesia. Use of paracetamol, NSAID and opioid-class medication were 21.2%, 18.2% and 9.09% respectively.

Conclusion: Our study demonstrates ongoing use of non-opioid-class medications, but a decrease in opioid usage in PWHA who received emicizumab prophylaxis.

Survey outcome of immunisation route preference for persons with haemophilia in Australia

Wong J[1], Parikh S[2], Carter T[1]
[1]Perth Children's Hospital, Perth, Australia, [2]Australian Haemophilia Centre Directors' Organisation (AHCDO), Malvern, Australia

Background: Australians are recommended to receive routine, non-covid immunisations through the intramuscular route, though immunisations are also administered subcutaneously in Persons with Haemophilia (PwH) to minimise the risk of intramuscular bleeding. Previous studies have shown that the immune system may not respond as well when a vaccine is administered subcutaneously.

Aim: To review preferred route of immunisation (subcutaneous vs intramuscular) for paediatric and adult PwH in Australia.

Method: An online survey was distributed to Haemophilia Treatment Centre (HTC) Directors across Australia. 14 HTCs completed the survey (8 paediatric, 5 adult and 1 HTC treating both, paediatric and adult PwH).

Result: Of the 14 HTCs, 10 prefer to administer immunisations subcutaneously (including 5/8 paediatric, 4/5 adult and 1/1 both). Overall, most HTCs prefer to administer immunisations subcutaneously for moderate and severe PwH, though higher preference was noted in PwH on demand treatment (8/14 on prophylaxis compared to 10/14 on demand), and intramuscularly for mild PwH (9/14). HTCs evenly prefer either route of vaccine administration for PwH on extended half-life or emicizumab treatment. However, most HTCs (7/10, excluding 4 HTCs that provided no response) prefer to administer immunisations subcutaneously for PwH on standard half-life treatment.

Conclusion: Australian HTCs prefer to administer vaccinations subcutaneously for PwH, particularly moderate to severe disease. There is a need to further investigate the efficacy of subcutaneous immunisation and broadly examining the adverse effects of both, subcutaneous and intramuscular immunisations.

References

- Bertamino M, Riccardi F, Banov L, Svahn J, Molinari AC. Hemophilia care in the pediatric age. Journal of Clinical Medicine. 2017 May 19;6(5):54.
- Carpenter SL, Soucie JM, Presley RJ, Ragni MV, Wicklund BM, Silvey M, Davidson H, Hemophilia Treatment Center Network Investigators. Hepatitis B vaccination is effective by subcutaneous route in children with bleeding disorders: a universal data collection database analysis. Haemophilia. 2015 Jan;21(1):e39-43.
- Cook IF. Subcutaneous vaccine administration–an outmoded practice. Human Vaccines & Immunotherapeutics. 2021 May 4;17(5):1329-41.

Quantitative pharmacokinetic model to characterize and extrapolate long-term FVIII activity levels in patients with severe hemophilia A treated with valactocogene roxaparvovec

Wong S[1], Agarwal S[2], Tiede A[3], Robinson T[2], Henshaw J[2]
[1]BioMarin Pharmaceutical Australia Pty Ltd, Crows Nest, Australia, [2]BioMarin Pharmaceutical Inc, Novato, United States, [3]Hannover Medical School, Hannover, Germany

Aim: The aim is to characterize the long-term trajectory of transgene-derived FVIII activity using a linear mixed effects (LME) model to estimate mean and median FVIII activity levels 5 years post-infusion.

Method: In GENEr8-1, an open-label, single-arm, multicenter phase 3 trial, 134 participants with severe hemophilia A received a single $6x10^{13}$ vg/kg dose of valoctocogene roxaparvovec. FVIII activity was assessed using the chromogenic substrate assay and one-stage assay. A previously published quantitative pharmacokinetic (PK) model was updated to extrapolate FVIII activity levels to 5 years post-infusion. Ln-transformed FVIII activity values from week 76 to 104 were fit to the LME model with random effects for participants on slope and intercept using a restricted maximum likelihood method with the lmer package in the R statistical computing software. The precision of parameter estimates and model diagnostics were evaluated to confirm goodness-of-fit. The model and extrapolation approach was further qualified by comparing to observed FVIII activity at week 156.

Results: The final LME model dataset included 928 observations from 120 participants. The long-term trajectory of FVIII activity was consistent with first-order elimination kinetics starting at week 76. Model parameter estimates were consistent with the previously published model & diagnostic plots showed no major deficiencies. FVIII activity was extrapolated to 5 years post-gene transfer (**Table**). Mean and median FVIII activity extrapolations at week 156 were consistent with observed values, confirming adequacy of the model.

Conclusion: Pharmacokinetic modeling indicates valoctocogene roxaparvovec-derived FVIII activity levels will remain in the mild hemophilia range for ≥5 years post-gene transfer for the majority of patients treated.

TABLES

Table. Extrapolated FVIII activity for GENEr8-1 $6x10^{13}$ vg/kg participants

	FVIII per CSA, IU/dL Mean ± SD	Median (min, max)
Week 104	22.3 ± 29.9	11.2 (BLQ, 173)
Week 156	17.2 ± 25.4	8.8 (BLQ, 160)
Week 208	13.8± 22.9	6.4 (BLQ, 149)
Week 260	11.6 ± 21.3	5.0 (BLQ, 139)

BLQ, below the limit of quantitation; CSA, chromogenic substrate assay; FVIII, factor VIII; SD, standard deviation

Post thrombotic syndrome in idiopathic upper extremity deep vein thrombosis

Yuen H[1,2], Ali S[3], Tran H[1,2], Curry G[1], Vanhaltren K[1], Coombs P[1], Gardiner E[3], Chunilal S[1,2]
[1]Monash Health, Clayton, Australia, [2]Monash University, Clayton, Australia, [3]Australian National University, , Australia

Introduction: Post thrombotic syndrome (PTS) is a feared complication of idiopathic upper extremity deep vein thrombosis (IUEDVT). However, the modified Villalta scale (MVS) used for PTS assessment has not been extensively validated in IUEDVT and currently there is no patient self-assessment tool for UEDVT PTS.

Aim: To explore rates of PTS and assess quality of life (QoL) and disability following IUEDVT treated with anticoagulation alone. To develop a patient-reported modified Villalta scale (PRMVS) and compare with MVS.

Method: We recruited patients who were at least 6 months post symptomatic IUEDVT involving the subclavian vein and treated with anticoagulation alone from Monash Health, a tertiary health network in Melbourne, Australia. We collected demographic data, assessed PTS using MVS and PRMVS and compared individual scores by Spearman correlation. We measured QoL (36-Item Short-Form Health Survey [SF-36], VEINES-QoL/Sym, a venous disease-specific QoL measures) and disability (Disabilities of the Arm, Shoulder and Hand (DASH) score). Results were compared in patients with and without PTS using *t*-tests.

Results: Our 23 patients (age 33.9 years, 39% male) were 26 months from IUEDVT diagnosis and received 7.1 months anticoagulation (range 3 months-ongoing). PTS diagnosed by MVS was present in 15 (65% 95%CI43-84) and by PRMVS in 10 (45% 95%CI24-68) with moderate correlation, r=0.69, *p*=0.0004, Figure 1. There were no cases with severe PTS by MVS whereas PRMVS deemed 2 (9%95%CI 1-29) as having severe PTS. We found no difference in QoL scores in patients with and without PTS but greater disability in patients with PTS than those without (DASH score MVS 11.96vs2.71 *p*=0.07, PRMVS 14.92vs2.83, *p*=0.04).

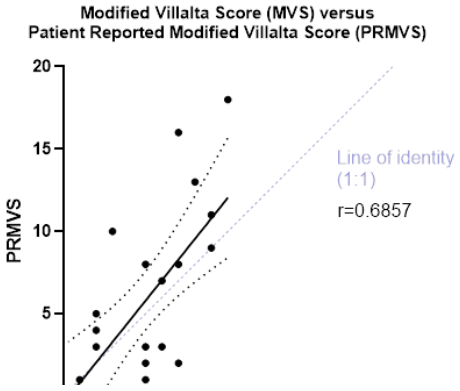


Figure 1. Scatter plot of the modified Villalta scale (MVS, X-axis) and the patient reported modified Villalta scale (PRMVS, Y-axis).

Conclusion: The PTS present in 45-65% of patients post IUEDVT was mostly mild to moderate and caused greater disability, but not lower QoL compared to patients without PTS. PRMVS shows moderate correlation to MVS and is a promising PTS self-assessment tool.

Assessing the needs of people affected by myeloma in regional, rural and remote settings.

Clarke T[1], Keogh J[1]
[1]***Myeloma Australia, The Gap, Australia***

Aim: To better understand the needs of people living with myeloma in regional, rural and remote (RRR) areas to improve the delivery of information and support programs.

Method: People living with myeloma in (RRR) areas of Australia were invited to participate in a 16-question survey to assess their information needs. Apart from demographic queries, most questions were open ended requiring written responses. A total of 181 responses were collected with an average of 150 responses per question.

Results: The survey had a national reach with participants spread across different states according to national statistics. Similarly, distances to the nearest capital city were reflective of national statistics. Approximately half of patients were treated locally, and half had to travel to a major centre, with some patients having a mixture of both. 83% of respondents were patients with myeloma, 13% partners, or carers and on 4% outside of these parameters.
Key Themes identified were 1) financial toxicity, as a result of having to travel to a specialist centre; 2) feelings of isolation and a desire for connection to others affected by myeloma 3) a prevalent belief that local healthcare professionals would benefit from myeloma specific education.

Conclusion: This survey has provided valuable insight into the experience of those individuals living with myeloma in RRR areas of Australia and has led to the formation of a virtual support group for this population and the expansion of our myeloma nurse development program which educates and supports nurses in these areas, to support and improve outcomes for people living with myeloma.

Home is Best: Patient Experience of Carfilzomib at Home Program

Combe N[1], Joyce T[1], Furphy E[1], Beer H[1], Khot A[1], Harrison S[1]
[1]*Peter MacCallum Cancer Centre, Parkville, Australia*

Home is Best: Patient Experience of Carfilzomib at Home Program

Introduction / Aim: Carfilzomib is an intravenous proteasome inhibitor used to treat relapsed/refractory myeloma. Given until progressive disease or unacceptable toxicity, it has a median PFS of 18 months thus requiring many hospital visits.

The Carfilzomib at Home Program was established with the Myeloma and Peter Mac@Home teams and this study aims to analyse the patient experience, patient reported outcome measures (PROMs) and acceptability of the program. Program rollout coincided with the start of the SARS-CoV-2 pandemic.

Method: A Carfilzomib at Home Patient Experience Questionnaire was co-designed with twenty-two questions developed to understand the patient experience of carfilzomib in hospital and home. Selected PROMs included EORTC QLQ-C30, MY20 and COST FACIT-Version 2.

All patients receiving in the Carfilzomib at Home Program between March 2020 to May 2023 were screened, and those still living were approached to participate. Study documents and questionnaires were sent, with consent implied on their return.
Descriptive data analysis includes the patient numbers of those who received carfilzomib at home, were approached to participate, completed the questionnaires and patient experience, PROMs, patient demographics, and treatment information.

Results: During the study timeframe, 29 patients received at least one carfilzomib infusion at home, 20 were able to participate; 14 consented, 4 declined and 2 were uncontactable. Of the 14 who received questionnaires, 10 completed, 2 withdrew and 2 remain pending.

Preliminary results show an average of 28 carfilzomib doses were administered (range 9 to 48); with an average of 10 doses in hospital (range 0 to 37), and 20 doses at home (range 3 to 40). Preliminary data indicates that at home delivery was very convenient, patients felt very confident and supported and having treatment at home was very important.

Conclusion: Preliminary results show that the Carfilzomib at Home Program is feasible and acceptable and valued highly by patients.

Co-designing a pilot patient support program (PSP) for a multiple myeloma (MM) therapy prior to listing on the Pharmaceutical Benefits Scheme

Furphy E[1], Beer H[1], Jones L[1], Davidson G[2], O'Donell N[2], Lorensuhewa N[3], Farrell J[3], Etto T[3], Robbins M[3]
[1]Myeloma Australia, Burnley,, Australia, [2]Atlantis Health Australia Pty Ltd, Macquarie Park,, Australia, [3]Antengene (AUS) Pty Ltd, Melbourne,, Australia

Aim: Myeloma Australia (MA), a non-profit support organisation, identified poor uptake of traditional pharmaceutical company facilitated patient support programs (PSPs) when the program is designed with a single drug focus and does not follow the patient beyond progression. MA together with Antengene (AUS) Pty Ltd, a biopharmaceutical company, co-designed a PSP for patients with relapsed/refractory MM (RRMM) who were prescribed XPOVIO® (selinexor) in combination with dexamethasone (Xd). Given that XPOVIO is a novel anti-myeloma drug available in Australia, the PSP aimed to enhance patient education and support their hospital-based treating teams for achieving optimal patient outcomes.

Method: Review of existing PSPs was conducted, including multi-disciplinary advisory boards including haematologists, nurses and pharmacists. MA and Antengene worked together with a behavioural change organisation, Atlantis Health, to review patients' key needs at the time of XPOVIO® therapy. The program designed would support patients at key time points throughout their treatment and beyond, that would be complimentary to the care provided by their hospital-based treating team.

Results: 14 RRMM patients (8 male and 6 female) prescribed Xd via an access program were enrolled into the X-TEND program (age range 47-85 yrs). Patients received contact from a specialist MA nurse at baseline and at defined intervals. Average call duration was 20 mins. Topics discussed included management of adverse events, general physical wellbeing, and psychosocial support. Average time on XPOVIO was 141 days and patients could remain on the program once therapy has ceased.

Conclusion: X-TEND is a comprehensive PSP that provides one-on-one bespoke support to patients on Xd regimen. It provides education on side-effect management, ensures the patient has a better understanding of their disease and offers unlimited emotional and psychosocial supportive care.

The PSP has filled gaps that the hospital system is not able to fill. MA has plans to replicate this model with other myeloma therapies.

The physical and psycho-social late effects of Adolescents and Young Adults (AYA) identified at first visit to an adult long term follow up (LTFU) clinic.

Garcia T[1], Panek-Hudson Y[1], Ritchie D[1], Bajel A[1], Routledge D[1], Cummins K[1], Koo R[1], Tan S[1]
[1]Department of Clinical Haematology and Bone Marrow Transplant Services, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Melbourne, Australia

Background: Approximately 15% of AlloBMTs performed in Australia each year are in children[2]. The number of AYA AlloBMT survivors is increasing due to advances in treatment modalities and supportive care. Prior treatment, conditioning therapy and transplant related complications are associated with a variety of late effects which impacts the quality of life of this group of patients[1]

Aims:
1. To describe common patient reported outcomes as reported by AYAs at first adult LTFU visit.
2. To describe common late effects experienced by AYAs identified at first adult LTFU visit.

Method: 83 AYA AlloBMT survivors were referred to the adult LTFU service from Nov 2014 to April 2023. AYAs account for 13% of the total number of adult LTFU referrals. Questionnaires addressing physical and psycho-social domains were sent to AYA patients to complete prior to or on the day of LTFU review.

Results: Data analysis at first adult LTFU visit show 93% of AYAs attended their appointment. 58% were transplanted for a haematological malignancy, 35% using a sibling donor and the majority received a non-TBI based conditioning regimen. 53% completed and returned patient questionnaires.

Table 1: Common late effects experienced by AYAs

Graft versus Host Disease (GvHD)	Acute – 28%
	Chronic – 12%
Cardiovascular disease	14%
Reduced bone density	27%
Female - post menopausal	18%

Table 2: Common patient reported outcomes reported by AYAs

Work and financial stressors	41%
Fatigue	41%
Fear/worry	27

Conclusion: The number of AYAs who transition to adult LTFU review continues to rise. The results show an excellent number of AYAs who attended their first LTFU review. It is important to keep this group of patients engaged in attendance at LTFU reviews to support appropriate evidence-based surveillance, education on risk reduction, and psycho-social support strategies to optimise patient outcomes.

References

- Diesch-Furlanetto, T. G.-S. (2021). Late effects after haematopoietic stem cell transplantation in ALL, long-term follow-up and transition: A step into adult life. *Frontiers in Paediatrics, 9*, 1-18. doi:10.3389/fped.2021.773895

1. Panek-Hudson, Y. (2021). The TransAllo: factors influencing attendance at and experiences of a long-term follow-up clinic post-allogeneic bone marrow transplant for patients transitioning from paediatric to adult services. *Australian JOurnal of Cancer Nursing, 22*(2), 11-17. doi:https://doi.org/10.33235/ajcn.22.2.11-17

Working Together! Treating Acute Promyelocytic Myeloid Leukaemia in the Community

Hill J[1], Finselbach R, Wills M, **Murray S**
[1]*View Health Chemo@Home, Bendolba, Australia*

Aim: To successfully collaborate care between a community chemotherapy service and a hospital day unit to treat a patient with APML in the consolidation phase of their treatment.

Background: The patient was a 38-year-old female, diagnosed with Acute Promyelocytic Myeloid Leukemia (APML) in April 2022. APML is an aggressive subtype of acute myeloid leukaemia accounting for 10% of all adult AML. Recommended treatment is an intensive regime, requiring inpatient care. Induction regime of prednisone days 1-10, Tretinoin (ATRA) days 1-36, Idarubicin days 2,4,6,8, and Arsenic trioxide days 9-36. Being high risk APML, 4 26-day cycles of Arsenic and ATRA were planned. Traditionally, this treatment would be given in hospital 5 days per week in a chemotherapy unit over at least 3 – 5 hours.

Started in 2013, View Health chemo@home founded the private chemotherapy at home market in Australia and remains the largest and most experienced provider operating in New South Wales, Queensland, Western Australia, South Australia, and Victoria.
The Calvary Mater Newcastle outpatient haematology day ward is the only public haematology unit in Newcastle, servicing most patients in the Hunter New England Area Health Service.

Method: Effective communication between health services was critical for successful home treatment. Administering Arsenic trioxide over an extended period requires close clinical monitoring including daily bloods to ensure Potassium and Magnesium levels are maintained at the required threshold, and twice weekly electrocardiograms to ensure QT interval is not prolonged due to electrolyte imbalance. The logistics of supplying and storing the Arsenic Trioxide with a short expiry period of 72 hours was another consideration.

Results: Safe and effective team nursing practice was maintained between the hospital and community settings. Uncomplicated completion of four consolidation cycles.

Conclusion: A successful collaboration between a community chemotherapy service and a hospital day unit to treat a patient with APML during their consolidation phase of their treatment in the community. The patient received individualised, safe, and effective care in the comfort of her home. She reported feeling grateful and felt well looked after by both services.

Implementation and outcome of a dedicated haematology symptom and urgent review service (SURS) in a regional Victoria setting

Jewell J[1], Roscoe D[1], Chapman R[1], Adams B[1], Wright T[1]
[1]*Latrobe Regional Hospital, Traralgon, Australia*

Background: Individuals receiving systemic anti-cancer therapy (SACT) for haematological malignancies often have unique supportive care requirements including the management of cytopenias and more frequent blood product support as well as repatriation needs following intensive SACT in a metropolitan centre.

Aim: To address unmet needs of patients with a haematological malignancy receiving SACT through the establishment of a Haematology Symptom & Urgent Review Service (SURS) at Latrobe Regional Hospital (LRH).

Method: A haematology SURS followed the existing oncology SURC model of care, a phone triage service with haematology physician support. All patients with a haematological malignancy receiving systemic anti-cancer therapy (SACT) were provided with a brochure advertising the haematology SURS program. Direct referrals were also accepted to capture individuals receiving oral SACT. United Kingdom Oncology Nurses Society (UKONS) 24-hour triage tool was utilised to grade symptoms and formulate patient advice.
Data collection is a 12-month period from June 2022 to June 2023 using existing SURC access database including the number of haematology specific SURS calls in addition to the supportive care needs.

Result: Data is currently available for 10.5 months with 627 calls to the Haematology SURS. This is an increase of more than 1000%. The full 12-month data set will be presented including haematology diagnosis, SACT regimen, probable action without SURS, presenting complaint graded (UKONS), required communication between health care professionals, and advice given to consumer.

Conclusion: The implementation of a dedicated Haematology SURS demonstrated a clear unmet need reflected in the increased number of haematology specific calls to the dedicated SURS program. The expansion of the specialist haematology nursing staff has allowed improvements in patient care coordination between rural and metropolitan health care services. This includes increasing the volume of care coordination and supportive care services at the regional centre for individuals requiring intensive therapy in a metropolitan setting.

Selinexor supportive care guidelines: consensus best practice initiative from HSANZ Myeloma Specialists Practice Network (M-SPN)

King T[1,2], Jagger J[3], Woodrow C[4], Demajo J[5], Beer H[5,6], Klarica D[7], Snowden A[8], White K[2,9,10], Catamero D[11], Quach H[12,13]
[1]Institute of Haematology, Royal Prince Alfred Hospital, Sydney, Australia, [2]Cancer Care Research Unit, Faculty of Medicine and Health, The University of Sydney, , , [3]Cancer Services, Central Coast Local Health District, , , [4]Cancer Services, Pricess Alexandra Hospital, Brisbane, , [5]Peter MacCullum Cancer Centre, Melbourne, , [6]Myeloma Australia, , , [7]Alfred Health, Melbourne, , [8]Precision Haematology, Melbourne, , [9]The Daffodil Centre, The University of Sydney, a joint venture with Cancer Council NSW, , , [10]Sydney Local Health District, , , [11]Mount Sinai Health System, NY, USA, [12]Department of Haematolgy, St Vincent's Hospital, Melbourne, , [13]University of Melbourne, ,

Aim: Selinexor has a significantly different toxicity profile to other MM treatments. Patients receiving selinexor may be heavily pre-treated, with significant disease and treatment related morbidities. Early recognition and implementation of supportive care interventions is critical to minimise selinexor toxicities and reduce risk of discontinuation of therapy. The aim of this study was to provide guidance for nurses to deliver best supportive care to those receiving selinexor through development of living consensus recommendations.

Method: A Selinexor Expert Advisory Group (SEAG) was established. All available published research, including selinexor licensing clinical trial data was examined. Australian and international guidelines associated with selinexor, and cancer supportive care were reviewed. Draft consensus recommendations were developed, and a formal consensus process followed with SEAG. Draft recommendations were reviewed by an international MM nurse leader experienced in managing selinexor toxicities, a haematologist, and a nurse researcher prior to presentation to the M-SPN membership. Feedback from members was minor and incorporated into the final document.

Results: Consensus recommendations were**: Patient and carer education** prior to commencing treatment focusing on recognising and early reporting of symptoms, adherence to medications to reduce toxicities, concurrent supportive medications. **Prompt and proactive symptom management** in the first cycles can reduce risk of treatment discontinuation due to toxicity. **Prompt dose reduction** in presence of therapy-related adverse events (TRAEs) with a 'go slow and low' approach can help patients adjust to therapy. The recommendations include brief information about selinexor indication, dosing/reductions, TRAE incidence, and management of toxicities. Treatment checklist links to 3rd party consumer information and patient treatment previously developed by our group.

Conclusion: Such level IV evidence that provides grade C recommendations provides useful evidence where data is lacking. This consensus guideline is important particularly as selinexor is reimbursed for the treatment of RRMM in Australia and will be updated regularly.

Multiple myeloma nurse practitioner models of care

Klarica D[1], Spencer A[1,2], Kalff A[1], Wright T[1,3]
[1]Alfred Health, Melbourne, Australia, [2]Monash University , Melbourne , Australia , [3]Latrobe Regional Hospital , Traralgon , Australia

Aim: To describe two Multiple Myeloma (MM) Nurse Practitioner (NP) led clinic models of care

Method: The model of care of the NP-led Monoclonal Gammopathy of Undetermined Significance (MGUS) and acute post-Autologous Stem Cell Transplant (ASCT) clinics are described. Numbers of patients and their demographics as well as identified improvements to patient care through NP-led clinics are presented.

Results: The NP-led MGUS clinic operates within the weekly Plasma Cell Disorder (PCD) clinic, which incorporates clinical mentorship through a post-clinic review meeting attended by expert physicians experienced in MM. The NP-led 3-month post-ASCT clinic was established in 2023 within the Haematology Supportive Care (HSC) program.

The NP-led clinic model of care provides a patient centred attention to understanding of disease and management and monitoring of symptoms and investigations.

Figure 1 shows the number of patient encounters at the NP-clinic by year with a total of 467 and 10 in the MGUS and post-ASCT clinics respectively. Table 1 summarises the demographics. Patients were predominately male, 64% and 60% in the MGUS and post-ASCT clinic respectively with a median age of 65.5 years in the MGUS clinic.

Table 1: Clinic demographics

	Total	Male n (%)	Female n (%)
MGUS	467	298 (64%)	169 (36%)
Median age at review (yrs) [range]		65.5 [35-96]	65.5 [36-95]
Post-ASCT	10	6 (60%)	4 (40%)
Median age at review (yrs) [range]		61.5 [55-68]	57.5 [51-64]

A request for further education and understanding of their condition is a common unmet need in the MGUS clinic. Post-ASCT patient concerns include slow gastrointestinal recovery with lagging nausea and bowel recovery, fatigue with lack of energy and emotional and financial burden with inability to return to work when needed.

Both clinics provide safe and patient centred supportive care improving appropriate use of limited medical specialist clinics and NP efficiency with a reduction in adhoc patient phone calls, emails and text messages.

Conclusion: Two effective NP-led models of health care delivery are described, addressing patients' concern in a safe, supported patient-centred program incorporating measurable outcomes to inform quality improvement programs.

The first year of extracorporeal photopheresis (ECP) at the Royal Brisbane & Women's Hospital: The ECP series

Mudie K[1]
[1]*Royal Brisbane & Women's Hospital, Herston, Australia*

Introduction: ECP is a recognised treatment for advanced stage erythrodermic cutaneous T-cell lymphoma (CTCL) and steroid refractory chronic-graft-versus-host-disease (cGVHD). In 2022, RBWH became the first hospital in Queensland to introduce this service. ECP is a leukapheresis based treatment, involving ex vivo treatment of lymphocytes with methoxsalen and UVA light. Treated cells are then re-infused to the patient. Apoptosis of treated cells occurs which has an immunomodulatory effect in vivo.

Aim: This case series will describe a series of patients, diagnosed with CTCL or cGVHD, who attended RBWH for ECP treatment during the first year of this new service. All patients treated had advanced stage disease and were refractory to multiple lines of therapy. Stage of disease before ECP commencement, treatment outcomes and ECP related adverse events will be discussed.

Method: Between October 2022 – October 2023, a total of 6 patients who commenced ECP therapy at RBWH, were included in this case series: 3 patients with CTCL and 3 patients with cGVHD. Prior to commencing ECP, all patients were assessed and met strict definitions of refractory disease according to the Medicare Benefits Schedule (MBS) definition. ECP treatment regimen was dependent upon disease being treated.

Results: Response to treatment was variable and was evaluated by clinical assessment, assisted by medical photography and/or disease specific grading tools. Only patients who demonstrated a response to the initial phase of treatment, as defined by the MBS, were eligible to proceed to a continuing phase of therapy.

Conclusion: Advanced stage erythrodermic CTCL and steroid refractory cGVHD are associated with reduced quality of life, and increased morbidity and mortality. Treatment continues to be challenging. A variety of ECP treatment regimens can be found in the literature, however high-level evidence for optimal treatment regimens is scarce. The introduction of ECP to RBWH provides an additional treatment option for this complex group of patients.

Chasing grannies: directed granulocyte donations for sepsis in severe Aplastic Anaemia at the Royal Hobart Hospital

Sheldon-Collins G[1], Blain Z[1], Hilder B[1], Hughes T[1], Montgomerie K[1], Pohle J[1], Prall R[1], Ragg S[1], Sih K[1], Smith M[1]
[1]Royal Hobart Hospital, Hobart, Australia

Aim: Directed granulocyte donations were required for a thirty-year-old woman with severe sepsis due to a persistent pelvic collection following fertility preservation. Despite treatments including transfusions of Lifeblood granulocytes transported from Melbourne she remained profoundly neutropenic and critically unwell. Transferring her was not possible, therefore collecting apheresis granulocytes from local volunteers became necessary. We outline the resources required, the challenges and outcomes.

Results: The directed granulocyte donations and transfusions achieved a positive clinical result. After lengthy hospitalisation including surgical interventions and care in the Intensive Care Unit, the patient is attending Royal Hobart Hospital as an outpatient. An Allogeneic Bone Marrow Transplant is being considered. Collaboration included Intensive Care, Day Chemotherapy, Oncology Inpatient and Radiation Therapy Units, Bone Marrow Transplant coordinators, pathology, pharmacy, and psychologist. A chain of custody enabled transport of the apheresis product via various departments to the ward.

Twelve donors were assessed. Two were immediately deferred due to unsuitability. Nine individuals donated twenty-three transfusions. The recipient received two to three transfusions weekly for approximately ten weeks. The donors were associated with the recipient. A third-party haematologist was appointed, and our psychologist was briefed and available for donor support.

Our centre is now developing clinical practice guidelines for the collection and transfusion of apheresis granulocytes.

Conclusion: Our experience demonstrates that directed apheresis granulocyte products can be safely and effectively collected and transfused in a regional tertiary hospital with a stem cell laboratory, apheresis, and radiation therapies on-site. The clinical team were impressed by the patient's resilience. We attribute her ongoing quality of life and psychological wellbeing to clinical care and the intangibles provided by her supportive home situation.

Optimisation of the Myeloma Clinical Nurse Coordinator role through the establishment of a formal myeloma Nurse-lead clinic

Wells (Jones) L[1], Taylor-Marshall R[1], Quach H[1], Mcpherson J[1], Barton A[1], Lukose M[1], Robertson J[1]

[1]*St Vincent's Hospital Melbourne, Fitzroy, Australia*

Aim: To improve equitable access to a myeloma clinical nurse coordinator (M-CNC) for all myeloma patient's, and make the contact that occurs more meaningful in multidisciplinary management.

A Major Hospital in Melbourne prides itself on delivering high quality patient centred care to people with Myeloma. The M-CNC plays a valuable role within the multidisciplinary team. During a review of referrals and contacts, issues around referral, visibility and usability of their service were recognised.

Method: The M-CNC established the existing essential contact time points. The evidence confirmed these, and highlighted areas for additional intervention, particularly in preventative and supportive care. A review of time points took place with the medical haematology team.

Focus groups were held with chemotherapy day unit nursing staff to establish an ideal referral method, documentation location, and to identify current gaps or overlapping areas of patient intervention.

The M-CNC team lastly worked alongside administration and finance to set up a formal nurse-lead clinic. It was incidentally identified that missed opportunities for billing of their service was occurring.

Results: A formal Myeloma Nurse Lead Clinic model was established within the organisation. Key benefits of the new model include:

- New standardisation of referral time points.
- Addition of new time points.
- New referral method.
- Improved visibility of a contact
- Reduction in missed or doubling of intervention.
- Improved usability of the documentation and plan.
- New revenue occurring, in which the myeloma nursing team has been able to access to further improve their EFT, resulting in more equitable access to their service.

Conclusion: A formal M-CNC nurse-lead clinic model which incorporates standardised referral at key time points allows for M-CNC intervention to occur when its most needed by patients. Improvements were also noted in referral rate, visibility, and usability of intervention. Positive financial implications occur for potential scalability of a CNC service - improving equitable access.

Transition from Albumex®20 to Alburex®20 AU in a Cellular Therapy Unit

Parsons E[1], Swain M[1], Mouminoglu A[1], Zhou J[1], Ho B[1], Bourke C[1]
[1]*Alfred Health, Melbourne, Australia*

Aim: Albumex®20 (20% albumin) is currently used by the Cellular Therapy Unit at the Alfred Hospital as a component of the cryoprotectant mixture used to cryopreserve cellular therapy products. CSL Behring is undergoing a phased transition from Albumex®20 to Alburex®20 AU.

Alburex®20 AU is manufactured using an updated manufacturing process. According to the National Blood Authority Australia[7], the manufacturing change has resulted in some final product differences. The replacement Alburex® products are not an exact equivalent of the current Albumex® products.

To ensure the Alburex® products are appropriate for use when Albumex® products are discontinued, it is necessary to assess the risks involved with introducing Alburex® products and assess any impact on the quality of the cryopreserved products.

Method: The validation will be conducted as a prospective validation and consists of the following:

(a) Three controlled rate freezer runs using each program profile in routine use, with Alburex®20 AU used in lieu of Albumex®20 in the sample temperature probe material.
(b) Three HPC(A) collections cryopreserved in parallel, directly comparing Albumex®20 and Alburex®20 AU key quality criteria. Patients selected may be either autologous patients or related allogeneic donors. 5 ml of plasma depleted HPC(A) will be cryopreserved with Alburex®20 AU whilst the remainder is cryopreserved with Albumex®20 as per standard procedures.

Testing to occur on HPC(A) product containing Alburex®20 AU:

- Microbial contamination testing
- CFU-GM assay
- Cryovial Viable CD34 recovery and viability

Direct comparison with HPC(A) product containing Albumex®20 (standard procedure):

- Microbial contamination testing
- Cryovial Viable CD34 recovery and viability

Results: To be presented.

Conclusion: To be presented.

Conflict of Interest Statement

No conflict of interest to disclose.

---

7 https://www.blood.gov.au/Transition%20of%20Australia%E2%80%99s%20Domestic%20Plasma%20Products#overlay-context=file/switching-immunoglobulin-products-pamphletpdf , accessed 06 Jun 2023

Mobilisation and lenalidomide in myeloma

Welschinger L[1], Milton C[2], Janowski W[2]

[1]*Nsw Health Pathology, Waratah, Australia,* [2]*Calvary Mater Newcastle Hospital, Waratah, Australia*

Aim: Study effects of different mobilisation regimens and exposure to lenalidomide (len) before peripheral blood stem cell (PBSC) collection on collection volume, nucleated cell counts (NCC) and $CD34^+$ cells.

Method: 215 multiple myeloma (MM) patients that received an autologous PBSC transplant at the Calvary Mater Newcastle Hospital (Newcastle, Australia) between 2/2011 – 12/2022 were assessed by mobilisation regimens of filgrastim (G-CSF) alone or in conjunction with 1.5g/m^2 (low dose) cyclophosphamide (cyclo) +/- plerixafor and lenalidomide, the control group was G-CSF and 3g/m^2 (high dose). Descriptive statistics used for continuous variables, multivariate analysis by correlation and regression used to evaluate total collection volume, NCC and $CD34^+$ cells/µL. *P* ≤0.05 was considered significant.

Results: No significant difference in gender (38% female) or median age (60.5). Mean body weight in the high dose cyclo group was lower than other groups (*P*=0.05). Significant differences (*P*<0.05) between groups in collection totals and differences within groups in volume and CD34.

Mobilisation Group	***Patients***	***Volume* (mL)***	***NCC* (x10^6/mL)***	***$CD34^+$cells* (x10^6/µL)***
G-CSF	5	733	2022	1176
G-CSF+plerixafor	4	730	1917	2383
G-CSF; len-exposed	4	727	1822	1476
G-CSF+plerixafor; len-exposed	11	368	1289	4009
1.5g/m^2 cyclo	56	444	292	2380
1.5g/m^2 cyclo+plerixafor	28	510	475	2356
1.5g/m^2 cyclo; len-exposed	25	204	326	3352
1.5g/m^2 cyclo+plerixafor; len-exposed	17	452	613	3245
3g/m^2 cyclo	65	510	343	4103

****Median***

No significant correlation in the G-CSF mobilisation groups except in the len-exposed +plerixafor (*P*=0.05) with a positive correlation in volume, NCC and $CD34^+$ cell number. Both doses of cyclo had correlations in collection totals that were significant (*P*<0.05) with difference being a negative correlation between volume and $CD34^+$ cell numbers, this was highly significant in the cyclo 1.5g/m^2 + len-exposed group (*P*<0.01).

Conclusion: Our MM patients are mobilised with low dose cyclo after 4 cycles of lenalidomide; patients given 2 cycles of lenalidomide proceed to G-CSF alone. Switching to low dose cyclo has proven patient benefits of out-patient administration and lower incidence of neutropenic sepsis. Our results indicate no negative impact on mobilisation from this regimen. Patients exposed to lenalidomide had lower volumes and greater $CD34^+$ cell numbers when mobilised with low dose cyclo.

---

[i] Itzykson R, Thépot S, Quesnel B, Dreyfus F, Beyne-Rauzy O, Turlure P, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood. 2011 Jan 13;117(2):403–11.

[ii] Lamarque M, Raynaud S, Itzykson R, Thepot S, Quesnel B, Dreyfus F, et al. The revised IPSS is a powerful tool to evaluate the outcome of MDS patients treated with azacitidine: the GFM experience. Blood. 2012 Dec 13;120(25):5084–5.

[iii] Molga A, Wall M, Chhetri R, Wee A, Singhal D, Singhal N, et al. Geriatric Assessment in Older People with Myelodysplasia Is Predictive of Azacitidine Therapy Completion and Survival: A Prospective Interventional Study at the Royal Adelaide Hospital. Blood. 2018 Nov 29;132:3101.

[iv] Breccia M, Salaroli A, Loglisci G, Finsinger P, Serrao A, Alimena G. MDS-specific comorbidity index is useful to identify myelodysplastic patients who can have better outcome with 5-azacitidine. Haematologica. 2012 Feb;97(2):e2.

[v] Hasegawa, K. , Wei, A. , Garcia-Manero, G. , Daver, N. , Rajakumaraswamy, N. , Iqbal, S. , Chan, R. , Hu, H. , Tse, P. , Yan, J. , Zoratti, M. , Xie, F. & Sallman, D. (2022). P774: CLINICAL OUTCOMES ASSOCIATED WITH AZACITIDINE MONOTHERAPY FOR TREATMENT-NAIVE PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROME (HR MDS): A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS. HemaSphere, 6 , 669-670. doi: 10.1097/01.HS9.0000845980.88839.7d.

[vi] Molga A, Wall M, Chhetri R, Wee A, Singhal D, Singhal N, et al. Geriatric Assessment in Older People with Myelodysplasia Is Predictive of Azacitidine Therapy Completion and Survival: A Prospective Interventional Study at the Royal Adelaide Hospital. Blood. 2018 Nov 29;132:3101.

[vii] Mitnitski A, Collerton J, Martin-Ruiz C, Jagger C, von Zglinicki T, Rockwood K, et al. Age-related frailty and its association with biological markers of ageing. BMC Medicine. 2015 Jul 13;13(1):161.

[viii] Bellusci et al. Therapeutic potential of convalescent plasma and SARS-CoV-2 hyperimmune immunoglobulins against BQ.1, BQ.1.1 and XBB variants

[ix] ACTRN12620001249943 Intravenous immunoglobulin rich in neutralising antibodies to SARS-CoV-2 (COVID-19) as a passive immunity modality in healthy individuals: an open-label, active control Phase 1/2 Study