The Maddie Riewoldt's Vision Centre of Research Excellence in Bone Marrow Biology presents the:

2023 National Symposium on Bone Marrow Failure Syndromes

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ABSTRACT BOOK

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SPEAKER ABSTRACTS

(Program order)

Professor Rodrigo T. Calado

A new era in the treatment of aplastic anemia: novel drugs and strategies

In this presentation, I review the current therapy for patients with aplastic anemia. For decades, immune aplastic anemia has been treated with hematopoietic stem cell transplant with a full-matched sibling as donor or immunosuppressive therapy with anti-thymocyte (ATG) and cyclosporine. Significant advance has been made in both fronts in the recent years, changing the therapy choices for patients. ATG formulations have been compared and equine ATG has proven to result in better and more sustainable responses. The addition of eltrombopag to immunosuppression significantly improved hematologic responses, rivaling transplant outcomes as front line treatment. Additionally, transplant modalities using alternative stem cell sources are more efficient and safe and may be applied as front line or salvage therapy in some circumstances. Other small molecules have been experimented in vitro to treat immune and inherited aplastic anemia with encouraging results. Today, the most severe complication after immunosuppression is clonal evolution with peculiar genomics but biomarkers to predict this complication are scarce.

Ariel Simpson (PhD Candidate)

Analysing CRISPR base-editing efficiencies for Bone Marrow Failure targets.

Simpson, Ariel 1, Fairfax, Kirsten 2, Hewitt, Alex W 1,2

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2. Centre for Eye Research Australia, The University of Melbourne, Victoria, Australia

Aim:
To investigate the potential of base editing strategies as targeted molecular therapies for inherited bone marrow failure syndromes (BMFS).

Methods:
Using documented clinically relevant variation (ClinVar) single nucletotide polymorphisms (SNPs) with nearby PAM sequences we catalogued all those amenable to editing by adenosine base editors (ABE), and then overlaid these results with genes known to be involved in inherited BMFS. To model correction of bone marrow failure pathogenic variants a library was developed which contained, within a construct, both the pathogenic variant and a window of 100 bases of the genomic sequence surrounding it, together with an incororated guide to correct the mutation. The library was transduced into Jurkat T cells containing an ABE and subjected to positive selection to isolate cells with bone marrow failure mutations. Genomic DNA was extracted for sequencing and subsequent analysis.

Results:
Targeted correction of pathogenic variants associated with BMFS was observed at several loci, but editing efficiencies was highly variable (range from 0-100% editing at target site, mean = 4.72% , SD = 0.19).
Even within the one gene editing efficiency was highly divergent, for example we introduced 4 BRCA2 variants, and observed 71.4% editing at 5791C:T and 0% editing at the other 3 loci. Presence of off-target edits within the editing window was also analysed, and was also highly variable (range from 0-100% editing at off-target site, mean = 5.51%, SD = 17.10). 30 non-targeting guides were included to estimate the amount of background editing.

Conclusion:
Base editing strategies hold promise for developing precise and effective treatments. Comparitive analysis in additional cell lines as well as assessment of different base editors will contribute to a more comphrensive evalulation of base editing strategies for bone marrow failure. This will contribute to a greater understanding of using CRISPR tools in blood cells.

Dr Lorna McLeman

Precision gene editing for Fanconi Anaemia I: A tale of Men

Lorna M McLeman[1,2], Astrid Glaser[1], Lu Liu[2], Sophie Monks O'Byrne, Andrew Elefanty[2], Rachel Conyers[2], Andrew J Deans[1]
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Fanconi Anaemia (FA) is the most common inherited bone marrow failure syndrome. In children with FA, bone marrow failure (at a mean age of 8 years) can be rescued by bone marrow transplantation. However, transplant has a high risk of death for FA patients and accelerates secondary cancer onset due to toxic side effects of chemotherapy used in conditioning.
Gene therapy and gene editing are emerging as alternative near-future treatments for FA that would not have these toxicities. In my project I am optimising Prime Editing – a cutting edge gene editing technology which precisely edits genetic mutations making only single stranded DNA breaks. I aim to show that Prime Editing can be a less toxic alternative to bone marrow transplant for treatment of inherited bone marrow failure.

I will present improved methods for prime editing developed using K652 erythroleukemia suspension cells and hematopoietic CD34+ cells derived from induced pluripotent stem cells (CD34+ iPSC). My experiments adapt the GFP:BFP conversion assay (where editing converts two amino acids in enhanced green fluorescent protein (EGFP) to make it blue (BFP)) for use in assessing accurate Prime editing. Improvements and optimisations will be made by rapidly screening multiple approaches for increased blue (BFP+) cell production. I will then apply the optimised method to correct a patient FA mutation in CD34+ primary hematopoietic stem cells.

We have so far demonstrated that Prime Editors can be delivered as DNA, mRNA or ribonucleoproteins. Optimal delivery was by mRNA for which I have achieved over 80% cellular transfection. By extending this work to CD34+ stem cells I am evaluating the most efficient method for gene editing these cells. We will then explore the safety and efficacy of Prime Edited human CD34+ stem cells as a potential future therapy for FA and other genetic diseases.

Dr Astrid Glaser

Precision gene editing for Fanconi Anaemia II: A tale of Mice

Astrid Glaser,[1] Lu Liu,[1,2] Kirsten Fairfax,[3] Elissah Granger,[1] Debora Arcieri Casolari,[5] Richard D'Andrea,[5] Thomas Gonda,[5] Sandy Hung,[4] Wayne Chrismani,[1] Alex Hewitt,[3,4] Jörg Heierhorst,[1] Andrew Deans[1,2]
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We aim to develop new gene editing therapies for the most common inherited bone marrow failure syndrome, Fanconi Anaemia (FA). Because of the challenges in obtaining haematopoietic stem cells (HSCs) from FA patients, we have generated a novel mouse model with an editable human FA patient mutation (FancL^{TATdel}). Detailed characterisation revealed that FancL^{TATdel} mice display characteristics of human FA, including mitomycin c (MMC) sensitivity, and a reduction in bone marrow haematopoietic stem and progenitor cells (LSK+ cells, $p<0.05$).

We will use FancL^{TATdel} mice as a pre-clinical model for *ex vivo* gene editing therapy of primary HSCs. We initially tested Prime Editing, a technology where reverse transcriptase (RT) is fused to Cas9 nickase (Cas9n) to transcribe an RNA-encoded edit into target DNA. Preliminary results showed Prime Editing at the FancL^{TATdel} mutation restored FancL gene sequence and MMC resistance, albeit at low levels. To increase the editing rates, we have adapted two Cas9 variants with relaxed targeting requirements (SpG and SpRY Cas9). To improve prime editing delivery into HSCs, we split expression the Cas9n and RT components to make Prime Editors that are smaller for use in integration deficient lentivirus or as ribonuclear protein complexes. These approaches will allow us to deliver Prime Editing using methods that have already been proven safe and effective in the clinic for gene addition therapy in FA and expand the range of mutations that could be corrected in a future clinical trial.

To establish feasibility of a prime editing gene therapy for FA and other inherited bone marrow failure disorders we will assess engraftment and survival of edited FANCL^{TATdel} HSCs after transplantation into non-irradiated FANCL^{TATdel} recipient mice. The mice will also be an excellent tool for studying the safety and efficacy of other gene editing methods, including rapidly advancing in vivo gene editing tools.

Associate Professor Adam Nelson

Metabolic alterations in Fanconi Anaemia and potential therapeutic strategies

Nelson, AS

Aim:
To examine the metabolic alterations in patients with Fanconi Anaemia.

Methods:
Mass spectroscopy and small molecule profiling of patients blood and urine collected at various timepoints, along with indirect calorimetry testing.

Results:
Key alterations in the metabolism were identified in patients with Fanconi Anaemia, including increased levels of oxidative stress, butyrate metabolites and changes in hunger signalling (Ghrelin), corresponding to changes in caloric requirements.

Conclusion:
We have identified significant changes in the metabolism of patients with Fanconi Anaemia that gives us insight into their inability to gain weight, and offers potential therapeutic interventions.

Professor Graham Lieschke

Evaluating new severe congential neutropenia disease genes in zebrafish models

Lieschke, Graham J.
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Aim:
Severe congenital neutropenia (SCN) and related disorders are rare types of hereditary bone marrow failure (BMF) syndromes. For approx. 20% of these patients, the genetic cause is unknown. Genome-wide analysis can suggest candidate causative genetic lesions, but functional studies are needed to verify gene/disease associations. *In vivo* animal models are one such approach.

Zebrafish are an attractive model for whole animal studies, with highly-conserved genetic, molecular and cell biological processes of granulopoiesis. Working within international collaborations using multiple experimental approaches, we aim to use zebrafish reverse genetics to strengthen the functional evidence connecting mutations of novel genes to SCN pathogenesis.

Methods:
Disruptive loss-of-function alleles in candidate genes are created using CRISPR/Cas9 gene editing in zebrafish reporter lines, enabling easy enumeration of neutrophil numbers *in vivo*. Leukocyte numbers and phenotypes are enumerated in crispant zebrafish embryos, and in stable mutant lines recovered in subsequent generations.

Results:
Our studies have modelled classical SCN syndromes and supported the discovery of new SCN-causative genes. Recently, our work has provided zebrafish models implicating SRPRA and SRP19 mutations as a previously-unrecognised cause of SCN.

Conclusion:
The studies contribute to knowledge about SCN pathogenesis and the regulation of granulopoiesis, provide diagnostic certainty for patients, and potentially offer insights that may lead to personalised therapies for SCN subtypes.

Dr Katharine Goodall

Developing curative therapy for complex immune disorders caused by hypomorphic RAG1 mutations

Goodall, Katharine J[1,2], Yow, Raymond[1],Wijanarko, Kevin J, Liani, Oniko, Li, Jacky Y, Sarila, Gulcan, Stanley, Edouard G, Ng, Elizabeth S, Staal[3], Frank JT, Elefanty, Andrew G[1,2]
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[3]Department of Immunology, Leiden University Medical Centre, Leiden, The Netherlands

Aim:
Hypomorphic mutations in the *RAG1* gene affect the adaptive immune system, resulting in limited immunity, autoimmunity and granuloma development. Allogeneic stem cell therapy is an effective treatment for these disorders, but HLA-matched donors can prove difficult to source, and therapy often produces deleterious side-effects. Our project forms part of a international consortium that aims to treat children with hypomorphic *RAG1* mutations using autologous haematopoietic stem cells corrected by gene therapy.

Methods:
We will generate induced pluripotent stem cells (iPSCs) harbouring a selection of hypomorphic *RAG1* mutations to model aspects of the disease *in vitro*. Control and *RAG1*-mutant iPSCs will be differentiated into haematopoietic stem cell-like cells (iHSCs) before further differentiation into B and T cells *in vitro*, or transplantation into immune deficient mice to assess lymphoid development *in vivo*. *In vitro*-derived mutant iHSCs will be used as a facsimile for patient CD34-expressing cells to test and optimise gene therapy approaches to correct the *RAG1* mutations in patient CD34 cells.

Results:
We are generating iPSC cells carrying several *RAG1* hypomorphic mutations, chosen to represent a spectrum of disease severity. We are optimising *in vitro* T cell and B cell differentiation, and we can now detect T cell abnormalities associated with deletion of *RAG1*. We believe these will be useful platforms to also model hypomorphic *RAG1*-mutant cells and to optimise the gene therapy technologies prior to clinical application. We will evaluate differentiated cells phenotypically, transcriptionally and functionally to understand the complex pathogenesis of these disorders.

Conclusion:
This project aims to improve the treatment of children with *RAG1* hypomorphic disorders by applying corrective gene therapy to patient CD34-expressing cells. We will generate iPSC models to enhance our understanding of these complex diseases, and use these cells to optimise gene therapy.

Dr Kirsten Fairfax

Immunogenetics and its implications for bone marrow failure syndromes

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Aim:

We sought to use single cell sequencing of blood cells from hundreds of individuals to understand how genetic differences drive transcriptional differences in blood cells**.**

Methods:
We used Illumina GSA arrays together with imputation using the Michigan Imputation Server with Minimac4 and the HRC panel to determine the genotype at over 5 million SNPs with a minor allele frequency of greater than 0.05. We concurrently generated scRNAseq data from 1,449,385 cells using a pooled multiplexing strategy, this used imputed genotype to assign donor ID to each cell. We have used various classification tools, including Azimuth, to classify cells into 28 different cell types, dendritic cell subtypes were then merged due to low numbers, prior to determining eQTL within each cell type.
We have also performed differential gene analysis within cell types to understand more about the biology of blood stem cells and to understand the heterogeneity in gene expression in different cell types across hundreds of individuals.

Results:
We found 14,488 cis-eQTLs across 21 cell types. The majority of these eQTL were seen in a single cell type. Differential expression of genes was conducted both looking for transcripts uniquely expressed in a population across all individuals, as well looking at differential expression of genes in a cell by cell comparison, for example when comparing CD14 and CD16+ monocytes from across the 982 individuals we could detect 111 differentially expressed genes. In total we were able to uncover 831 statistically significant differences in gene expression in 25 cell types with an average expression change of 2-fold in a cell type of interest.

Conclusion:
Understanding how key immune regulatory and signalling pathways differ as a consequence of genotype may provide greater ability to tailor treatment.

Dr Vashe Chandrakanthan

Bioengineered Haematopoietic Stem Cells and Stromal Cells Re-establish Haematopoietic Stem Cell Niche in Failed Bone Marrow

Vashe Chandrakanthan[1]

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Haematopoietic stem cells (HSC) have extensive self-renewal potential and are the source of daughter cells that proliferate, mature, and develop into all types of blood cells. To date, however, long-term human HSCs cannot be expanded adequately *in vitro* without losing regenerative potential. Current gene editing techniques for human HSCs lose long-term stemness *in vivo*, limiting the application of this technology for durable cure of inherited haemoglobinopathies or bone marrow failure syndromes.

Clinically relevant long-term transplantable HSCs develop just once during embryo development. First HSCs appear at embryonic day 10.5 (E10.5) in murine and day 30 in human – directly from specialised endothelium called "haemogenic endothelium" that line the ventral surface of the dorsal aorta in a region known as the aorta-gonad-mesonephros (AGM). Accessory cells and soluble factors that regulate and determine the induction of haemogenic endothelium to produce HSCs in the AGM are not fully understood. Gaining insights into these events will help us to develop protocols that aim to generate HSCs from patient-specific endothelium for therapeutic use. We have discovered a population of PDGFRA^{+} stromal cells in the sub-endothelial wall of the aorta of AGM that are critical for the emergence of HSCs[1].

These PDGFRA^{+} AGM stromal cells (PSCs) developmentally derived from the mesoderm posterior 1 (*Mesp1*) and reside at the basal surface of the dorsal aortic endothelium upon HSC emergence at E10.5–E11.5. Later, at the E13.5 these *Mesp1*-derived (*Mesp1*^{der}) PSCs were replaced with *Wnt1*-derived (*Wnt1*^{der}) neural crest PSCs. Co-aggregating *Mesp1*^{der}PSCs with non-haemogenic adult– cardiac, lung, aortic endothelium resulted in the generation of long-term repopulating HSCs. *Mesp1*^{der}PSCs mediated endothelium-to-haematopoietic transition that generate HSC process was disrupted by dose-dependent inhibition of PDGFRA, NOTCH, BMP and WNT signalling.

Transplantation of adult endothelium derived HSCs with *Mesp1*^{der}PSCs into irradiation induced bone marrow failed mice led to restoration of long-term HSC niche with establishment of WNT signalling between HSCs and *Mesp1*^{der} stromal cells, which is important for HSC maintenance of self-renewal and differentiation. Taken together we report a translatable research outcome with high clinical precision and importance in the development of next generation HSC therapies.

Reference:

1. Chandrakanthan et al., Nature Cell Biology 2022.

Ryan Collinson (PhD Candidate)

Loss of TCF3 is associated with progression to bone marrow scarring and failure in myeloproliferative neoplasms.

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Aim:

Myeloproliferative neoplasms (MPN) are clonal haemopoietic disorders that generally commence with indolent disease and can progress to myelofibrosis (MF). This is thought to be due to dysfunctional megakaryocytes driving the fibrotic process and which leads to bone marrow failure. Since *TCF3* (DNA transcription factor) is essential for megakaryocyte development, we assessed whether there are acquired genomic and proteomic defects in these cells and their platelet progeny in MPN.

Methods:

Samples from a total of 116 MPN patients (85 indolent disease, 31 MF) were analysed in this study. Megakaryocytes were isolated from bone marrow of MPN patients underwent targeted next-generation sequencing (NGS). Platelets were assessed for gene expression by transcriptomic NGS and protein levels using mass spectrometry. Megakaryocyte TCF3 was assessed using bone marrow sections of MPN patients and controls.

Results:

TCF3 mutations were identified in megakaryocytes in 3 MF patients. These non-synonymous mutations were predicted to be pathogenic (MutationTaster2 mutation prediction software). Gene and protein expression analysis showed platelets from MF had a profile consistent with loss of *TCF3*, whereas platelets from patients with indolent MPN did not. In the bone marrow, there was reduced TCF3 in megakaryocytes in MF compared with both indolent MPN (padj=0.0433) and normal marrows. TCF3-negative megakaryocytes exhibited morphological features reminiscent of those characteristic in MF (i.e. pyknotic nuclei; adjacent to trabecular bone).

Conclusion:

In MF, there are *TCF3* mutations and reduced protein expression in megakaryocytes. There is also predicted loss of activity based on platelet gene and protein expression profiles. This suggests that *TCF3* inhibition could underpin the upregulation of genes and proteins associated with bone marrow fibrosis and failure in MPN. If confirmed in larger studies, drugs targeting the TCF3 network may provide a novel treatment strategy to avert marrow fibrosis and bone marrow failure.

Dr Lucy Fox

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

Fox LC[1]., Den Elzen, N[1]., Barth, L[1]., Man, K[1]., Panjari, M[1]., Pazhakh, V[1]., Tan, M.,[1] Thompson E[1]., Blombery P.[1]

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The bone marrow failure syndromes (BMFS) are clinically diverse, with both inherited and acquired etiologies. Deleterious germline variants conferring a susceptiblity 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 406 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 (36 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

Dr Sharon Savage

Cancer screening approaches in Fanconi anaemia

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Aim:
Individuals with Fanconi anaemia (FA), an inherited chromosome instability disorder, have exceedingly high risks of head and neck squamous cell carcinoma (SCC) (predominantly of the oral cavity), as well as oesophageal, vulvar, and anal SCC compared with the general population. In 2020, Velleuer et al (PMID: 32022466) reported the first FA oral SCC screening study using oral brush biopsy sampling of oral potentially malignant lesions (OPML) and found it to be highly sensitive and specific compared with histopathology of incisional biopsies, suggesting that this method could be incorporated as a screening strategy for oral SCC in FA. Since there are essentially no evidence-based screening or treatment guidelines for SCC in FA, this recently launched study seeks to establish the framework for cancer screening, prevention, and therapeutic trials through international collaborations and the Fanconi Anemia Research Fund (FARF) Cancer Consortium.

Methods:
This is a prospective cohort study involving questionnaires, clinical and research evaluations, clinical and research laboratory tests, review of medical records, and cancer surveillance (https://marrowfailure.cancer.gov/fanconi-anemia/, ClinicalTrials.gov identifier NCT05687149). Eligibility criteria include individuals with FA ≥ 12 years of age (aged 8 to 12 years if history of oral lesions or concerning symptoms) from North America and other countries provided they can travel to the USA on their own to the NIH Clinical Center, Bethesda, MD. Evaluations at the NIH will include blood and saliva collections, oral brush biopsies and oral photographs, as well as comprehensive assessments by otorhinolaryngology, dental, dermatology, gastroenterology, and gynaecology (for females). This study is designed to be an open-ended natural history study with anticipated enrolment of up to 200 participants over a five-year period with an enrolment of 20-30 new participants per year. Follow-up is planned for 10 years.

Results:
As of August 2023, 45 individuals have contacted us, 23 have completed the eligibility questionnaire,12 have been consented to participate and five have been evaluated at the NIH. Oral brush biopsies have been sent for cytology and DNA image cytometry to extramural collaborators. If needed, new cancer diagnoses will be presented at the FARF's tumour board.

Conclusion:
This international collaborative study with the FA Cancer Consortium will provide new information on OPML development and robustly quantify the risk of progression of OPML to cancer in FA. It will also identify potential precursor states for oesophageal and anogenital cancers in FA. This study will generate prospective data on approaches to detect precancers in individuals with FA and create the foundation on which to build prevention studies seeking to reduce morbidity and improve overall survival in FA.

Dr Diva Baggio

Approach to managing healthy individuals with germline predisposition to haematologic malignancy: The example of blood donation

Baggio, Diva[1], Fox, Lucy[2,3], Wood, Erica[3,4]
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Aim and background:
Germline predisposition to haematologic malignancy is increasingly recognised in healthy individuals through cascade screening. Such a diagnosis can carry implications for many aspects of a person's life, over and above their own health. However, how best to develop health policy and to counsel asymptomatic people about the potential impacts remains unknown. One example is whether blood donation is safe and appropriate. These individuals are often highly motivated to donate blood, having witnessed one or more family members develop haematologic malignancy. However, there are currently no peer-reviewed publications addressing this topic. Some people with a hereditary predisposition syndrome may have donated successfully for many years prior to genetic testing. Additionally, there is precedent in existing international blood donor selection guidelines to allow people with a history of cured malignancy or pre-malignant conditions to donate blood, due to a neglibile risk of cancer transmissability via allogeneic blood products. We describe an international project aiming to document current practices among transfusion services regarding blood donation by people with germline predisposition syndromes.

Methods and results:
As part of an International Society of Blood Transfusion "Vox Sanguinis International Forum", a standardised questionnaire was developed and distributed electronically. Questions pertained to blood donors with current and past (cured) solid organ and haematologic malignancy, as well as healthy individuals with germline predisposition to solid organ and haematologic malignancy. The presence or absence of pre-defined eligibility criteria for the donation of whole blood or fractionated products, perceived donor risks (e.g. exacerbation of cytopenias, adverse donor reaction) and recipient risks (e.g. cellular dysfunction in donor product, transmission of malignancy) and management of donations prior to diagnosis were recorded. The results will be published later this year.

Conclusion:
Blood services must balance the potential risks of blood donation and the demand for sufficient national blood supplies, and also provide counselling for potential, actual and deferred donors. As genetic screening for these syndromes becomes more widely available, establishing evidence-based guidelines for these individuals to donate blood is of increasing importance. The results of this survey will contribute to that effort.

Dr Eliska Furlong

A demonstrative case of high clinical utility of real-time data provided by prospective multimodal genomics study of inherited bone marrow failure and related disorders in a child presenting with myelodysplastic syndrome, congenital hearing loss, and unbalanced aberration der(1;7).

Eliska Furlong[1], Cathy Kiraly-Borri[2], Sarah O'Sullivan[2], Rebecca de Kraa[3], Dianne De Santis[4], Nicole Den Elzen[5], Lucy Fox[5,6], Piers Blombery[5,6], Shanti Ramachandran[1,7], Tina Carter[1,7,8]

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Aim:
A congenital bone marrow failure syndrome (BMF) associated with progression to myelodysplasia such as Fanconi anaemia or dyskeratosis congenita, or familial haematopoietic disorder, notably GATA2 or SAMD9/SAMD9L (SAMD9/9L), was highly suspected in this 10-year old girl presenting with hypocellular myelodysplasic syndrome (MDS), congenital hearing loss, and unbalanced der(1;7) resulting in loss of chromosome 7 q arm (7q-).

Methods:
A comprehensive diagnostic approach was undertaken at a local and national level to establish the diagnosis and management in this paediatric patient. To facilitate timely identification of a likely underlying genetic variant, the patient was enrolled in the Inherited Bone Marrow Disorders (IBMDx) study run by the Haematology team at Peter MacCallum Cancer Centre where whole genome and transcriptome sequencing are used in children and adults with suspected inherited BMF and related disorders.

Results:
The constelation of hypocellular MDS, congenital hearing loss and 7q- in a non-syndromic appearing 10-year old child was highly suggestive of GATA2 or SAMD9/9L familial haematopoietic disorder. Germline *GATA2* and *SAMD9/9L* variants are frequently associated with -7 or -7q, variable non-haematologic phenotype, and collectively represent at least half of all paediatric MDS cases with monosomy 7[1,2].

Unexpectedly, a germline heterozygous pathogenic variant was found in the *PTPN11* gene, establishing the diagnosis of Noonan syndrome (NS). NS is a mutlisystemic genetic disorder resulting from genetic alterations in RAS-MAPK pathway. Although NS has been associated with haematological cancers occuring during childhood such as juvenile myelomonocytic leukaemia (JMML), acute myeloid and B-cell leukaemia, and neonatal onset JMML-like myeloproliferative disorder[3]; *PTPN11* germline mutations have not been associated with childhood inherited BMF[4].

Following childhood MDS consensus approach[5,6], this patient proceeded to haematopoietic stem cell transplantation (HSCT) with her *PTPN11* wild type HLA-matched sibling; and has been taken up by appropriate services for NS life-long surveillance.

Conclusion:
This case highlights the benefits of real-time multimodal genomics data in the diagnosis and management of patients with rare inherited BMF disorders. Challenging decisions including proceeding to HSCT underscore the need for collaborative evidence-based approach in this rapidly expanding field.

References:
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2. Wlodarski MW, Hirabayashi S, Pastor V, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood* 2016; **127**(11): 1387-97; quiz 518.
3. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet* 2013; **381**(9863): 333-42.
4. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood* 2018; **131**(7): 717-32.
5. Locatelli F, Strahm B. How I treat myelodysplastic syndromes of childhood. *Blood* 2018; **131**(13): 1406-14.
6. EWOG-MDS/SAA Guidelines for HSCT in Childhood MDS and JMML. https://ewog-mds-saa.org/treatment.html.

Dr Michelle Tan

A novel germline SAMD9 variant in a paediatric patient with bone marrow failure, with longitudinal molecular analysis of a somatic rescue event

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Introduction:
Germline gain-of-function *SAMD9/SAMD9L* variants are increasingly recognised in inherited bone marrow failure syndromes (IBMFS) and myelodysplastic syndrome (MDS). Patients with germline SAMD9 variants frequently develop somatic rescue variants(1).

We describe a novel deleterious germline *SAMD9* variant and longitudinal molecular analysis of a somatic rescue variant, in a patient with IBMF.

Case Description:
A 12-year old female with BMF since early infancy attended for review. Her full blood examination showed a haemoglobin of 90 g/L, MCV 107fL, and platelet count of 34 x10^9/L. Leucocyte numbers were normal. Chromosomal fragility, telomere length and PNH testing by flow cytometry were normal. Bone marrow biopsies at 3, 7 and 9 years old demonstrated progressive hypoplasia. All marrow samples showed normal cytogenetics. Her medical history was significant for intrauterine growth restriction (IUGR) at 37 weeks gestation, membranous glomerulonephritis and reflux nephropathy.

Targeted next generation sequencing (NGS) on peripheral blood identified a novel missense heterogeous *SAMD9* variant (c.1940A>C; p.Asp647Ala) at germline variant allele frequency (VAF). A second truncating *SAMD9* variant (c.1030C>T; p.Arg334*) was present at 30% VAF. Germline origin of the p.Asp647Ala variant was demonstrated by Sanger sequencing performed on a buccal swab sample. The truncating variant was absent from buccal DNA, implicating it as a likely somatic rescue event. Segregation testing confirmed the germline SAMD9 variant (p.Asp647Ala) as de novo.

Retrospective NGS analysis was performed on serial DNA samples from the patient's stored peripheral blood and bone marrow (Table 1).

Age	Sample	Germline p.Asp647Ala VAF(%)	Somatic p.Arg334* VAF(%)
9 months	Peripheral blood	40	Not detected
13 months	Peripheral blood	43	Not detected
3 years	Peripheral blood	45	22
5 years	Bone marrow	45	30
9 years	Peripheral blood	46	30
10 years	Peripheral blood	47	22

To date, the patient's peripheral blood counts have remained stable and she is transfusion independent. She has been referred for allograft consideration.

Discussion:
First described in relation to the autosomal dominant MIRAGE syndrome(2) and subsequently in monosomy 7 MDS and leukaemia syndrome 2(3), germline *SAMD9* variants predispose to a heterogenous group of haematological and non-haematological manifestions. The IUGR and renal dysfunction in this case are features of MIRAGE syndrome. More recently, variants have been observed in non-syndromic bone marrow failure and MDS(4,5).
The recognised p.Arg334* variant is a compelling somatic rescue event(6), likely providing a protective mechanism in this patient. Serial somatic analysis in this case demonstrates clonal evolution under selective pressure of the germline SAMD9 variant.

Conclusion:
In patients with germline *SAMD9,* somatic monitoring in conjunction with intermittent assessment of peripheral counts and cytogenetics may be of benefit to detect loss of a compensatory somatic rescue variant or emergence of new clones with leukaemic potential.

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Rachel Edwards (PhD Candidate)

Optimising symptom management in young people receiving Bone Marrow Transplantation: Symptom-PROMPT.

Edwards, Rachel M1[1]

Aim:
Young people receiving Bone Marrow Transplantation (BMT) are at high risk of treatment-related symptom burden, potential for distress and disruption to daily life (Ullrich et al., 2018). Optimal symptom management is dependent upon accurate assessment and appropriate intervention (Dupuis et al., 2019). Despite overwhelming evidence to support the use of patient-reported outcome measures (PROMs) to assess and monitor symptoms, these resources are not well integrated or routinely used to inform clinical care in BMT services for young people (Johnston et al., 2018; Leahy et al., 2018; Withycombe et al., 2019).

This program of research aims to reduce symptom burden and improve quality of life in children treated with BMT by:
1. improving nursing knowledge, attitudes and behaviours of symptom management for children receiving BMT and
2. establishing the feasibility, acceptability and sustainability of routine use of a PROM in children's BMT services.

Methods:
This health services research project uses a hybrid type 2 effectiveness-implementation design using mixed methods to simultaneously evaluate the efficacy and implementation of the intervention in a pilot trial. There are three phases in this program of work being undertaken in collaboration with a quaternary paediatric Bone Marrow Transplant service.
1. Phase 1 - the adaptation and co-design of a suite of education resources to support nurses in symptom assessment and management
2. Phase 2 - the delivery and evaluation of the education resources on the effectiveness of training with pre and post evaluation of nursing staff knowledge, attitude and practice mapped to Kirkpatrick's 4-level model of evaluation.
3. Phase 3 - implementation of a PROM in the acute inpatient phase of BMT in a pre-post design comparing a control and intervention group.

Results:
The education resources and preliminary findings from Phase 1 and 2 will be presented along with the preliminary findings from the PROM data in Phase 3. This data will describe the patient symptom trajectory during hospitalisation for BMT.

Conclusion:
It is anticipated that the project will improve nurse knowledge attitudes and behaviours in symptom assessment and management and increase confidence in supporting patients with symptom distress. This project will provide new information about the trajectory of symptoms during acute BMT admission and will inform the appropriateness of symptom screening in BMT and recommendations for implementation into routine clinical practice. This information will be pivotal to upscale the model for application both nationally and internationally.

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Alice Maier

Utility of a neurobehavioral assessment for treatment planning, educational, and family support for children undergoing bone marrow transplantation for non-malignant disease.

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Aim:
Bone marrow transplantation (BMT) is increasingly being offered as a cure for children with bone marrow failure syndromes (BMFS) and other non-malignant conditions. BMT is associated with risks for child development, however, there is sparse research on the developmental, educational, and psychological outcomes in children undergoing BMT with non-malignant conditions. Study aims are to:

1. Develop and implement a pre-BMT neurobehavioural assessment within 12 weeks of transplant for patients with bone marrow failure syndromes (BMFS) or non-malignant conditions undergoing treatment at the Royal Children's Hospital (RCH), Melbourne.
2. Evaluate the utility of a novel neurobehavioural assessment and monitoring protocol from the perspective of, (i) parents, and (ii) healthcare providers.

Phase 1 of this study will be presented.

Methods:
This study will be conducted as a prospective cohort study. Phase 1 of the project is a qualitative study which will engage consumers (patients and caregivers) and BMT healthcare providers (HCPs). We will utilise focus groups and interviews to better understand cognitive/educational, psychological, social, and information needs of patients/families in the pre- and post-transplantation periods and map existing assessment and care pathways. Data obtained from Phase 1 will be utilised in the co-design of the neurobehvioural assessment protocol.

Results:
We will present preliminary qualitative findings from Phase 1 of this study examining the lived experience of BMT patients with BMFS and their caregivers and their views on how best to address their needs pre and post transplant. We will also present the perspectives of multidisciplinary BMT HCPs. Preliminary data will be analysed thematically utilised the approach of Braun and Clark.[1]

Conclusion:
Consumer and stakeholder engaagement is critical to the successful implementation of new clinical protocols. We will discuss the preliminary findings of this study with an emphasis on the role of consumer/stakeholder engagement and co-design processes.

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Dr Ashleigh Scott

Lower dose antithymocte globulin as frontline treatment for adult acquired aplastic anaemia.

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Aim:
Combination antithymocyte globulin (ATG) and cyclosporine A (CYA) is standard frontline treatment for newly diagnosed transplant-ineligible acquired AA; however, there are minimal data regarding optimum ATG dosing schedules. Our institution has historically employed lower-dose ATG (ATGAM 15mg/kg/day D1-5). We aimed to review local outcomes using this protocol.

Methods:
We performed a retrospective audit of all patients treated with first-line ATGAM+CYA between 2006-2022. Data regarding disease characteristics and outcomes were recorded, including incidences of response, relapse, and secondary malignancy. Disease severity and treatment response criteria utilised were those defined by Camitta and Killick respectively. Survival was calculated using Kaplan-Meier method.

Results:
During the 17-year period, 33 patients were treated and their data included for analysis. Median age at treatment commencement was 42 years (range 17-80 years), 11 had a detectable PNH clone, and 26 were severe (19) or very severe (7). Median time from diagnosis to treatment was 17 days. 19 (57%) patients achieved a partial (11) or complete (8) response, with median time to first response 68 days and best response 114 days. At 3, 6 and 12 months post-treatment, cumulative incidences of first response were 45%, 48%, and 58%, and for best response were 30%, 42%, and 55%.
In responding patients, 4 subsequently relapsed. Secondary MDS and/or AML occurred in 5 patients, including 3 responders and 2 non-responders. Allogeneic BMT was performed in 12 patients, for either non-response (6), relapse (3) or secondary malignancy (3) respectively. After a median study follow-up of 2.5 years (range 1 month to 12 years), 9 patients have died, due to either refractory AA (5), second malignancy (2) or post-BMT complications (2).

Conclusion:
Within the limitations of a single-centre retrospective study, our results suggest that lower dose ATGAM at 15mg/kg/day D1-5 produces similar responses and outcomes as published standard-dose ATGAM+CYA schedules.

Professor Erica Wood & Professor Melissa Southey

The Aplastic Anaemia and Other Bone Marrow Failure Syndromes Registry (AAR) and Australian Marrow Failure Biobank (AMFB)

Wood, Erica M.[1,8], Southey, Melissa C.[2,3,4,5], McQuilten, Zoe K.[1,8], Fox, Lucy C.[1,6], Shortt, J. [7,8] , Young L.[1], Fox V.[1], Sutherland R.[1], Tsimiklis H.[3], Waters N.[1], and Firkin F.[5] on behalf of the Australian Aplastic Anaemia and Other Bone Marrow Failure Syndromes Registry Steering Committee and Investigators, and the Australian Marrow Failure Biobank
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Background and Aim:
Registries are vital to understanding rare diseases, where it is difficult to conduct high-quality clinical trials. They provide important 'real world' data on epidemiology, current management and patient outcomes, and platforms to support a wide range of research, including health economics analyses and efficient registry-based clinical trials. Registry-linked biorepositories provide critical resources for laboratory research.

The Aplastic Anaemia and Other Bone Marrow Failure Syndromes (BMFS) Registry (AAR) was established in 2013 by Monash University in collaboration with hospitals across Australia. The Australian Marrow Failure Biobank (AMFB) was established in 2023 as a joint intiative between the AAR and Biobanking Victoria at Monash University, supported by Maddie Riewoldt's Vision. The AMFB is developing a biorepository of samples from patients with BMFS to complement the AAR clinical data, and coordinating the collection, processing, storage and distribution of matched tissue sample and datasets.

Methods & Results:
The AAR contains a comprehensive clinical dataset on 353 Australian adult and paediatric patients with BMFS from 32 institutions nationally; 7 additional sites have ethics/governance approval. Data are entered by sites at diagnosis, 6 and 12 months and annually thereafter, and stored in a secure Monash REDCap database. Data analysis and interpretation is undertaken by Monash staff and specialist clinicians on the AAR steering committee. The AAR supports the MRFF-funded DIAAMOND trial, and will enable long-term trial follow-up.

The AMFB received HREC approval in March 2023 and four adult and paediatric hospitals are participating in pilot activities. Storage and release of bone marrow, peripheral blood and germline samples is managed by Biobanking Victoria, overseen by a Data and Biologicals Access Committee.

Conclusion:
The AAR and AMFB are key resources for BMFS research in Australia, including the DIAAMOND trial. AAR clinical data and AMFB samples will be available to approved researchers working to identify new diagnostic and therapeutic strategies for BMFS

Associate Professor Stephen Ting

An update of the DiAAMOND-Ava-First and DiAAMOND-Ava-Next Bayesian Optimal Phase II Trials studying the Efficacy and Safety of Avatrombopag in Combination with Immunosuppressive Therapy in Treatment Naïve and Relapsed/Refractory Severe Aplastic Anaemia.

Stephen B Ting[1,2], Zoe K McQuilten[3,4], Lucy C Fox[4,5,6], Vanessa Fox[4], Stephane Heritier[4], Tracey J Batt[7], Piers Blombery[6,8], Ilona Cunningham[9], Jennifer Curnow[10,11], Alisa Higgins[12], Devendra Hiwase[13,14,15], Robin J Filshie[16], Frank Firkin[16], Paul Lacaze[12], Kylie D Mason[6,8], Anthony K Mills[17,18], Dominic Pepperell[19], William Stevenson[11,20], Jeff Szer[6,8], Neil Waters[4], Kate Wilson[11] and Erica M Wood[3,4].

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Aim:
The RACE trial shows immunosuppressive therapy (IST) with antithymocyte globulin (ATG), ciclosporin (Ci) plus eltrombopag (El), a thrombopoietin-receptor agonist (TPO-A), is the current standard of care for sAA patients not eligible or suitable for allogeneic stem cell transplant. Whilst the overall response in RACE was 68% at 6 months in the sAA cohort receiving ATG-Ci+El, of these only 32% achieved a complete response (CR) and a significant proportion were refractory or relapsed (R/R). Avatrombopag is a second-generation TPO-A, and like eltrombopag, lacks competitive binding with endogenous TPO, suggesting it may also be effective in AA. Avatrombopag has potential advantages over eltrombopag, including dosing, toxicity profile and pharmacokinetics. It also shows greater in vitro and in vivo pharmacological potency. To date, avatrombopag has not been studied in sAA.

Methods:
The DIAAMOND Ava-FIRST and DIAAMOND Ava-NEXT trials are investigator-initiated, non-randomised, single-arm registry-based Bayesian Optimal Phase II (BOP2) trials. The FIRST trial evaluates avatrombopag in addition to IST in untreated sAA patients. IST comprises horse ATG and ciclosporin with avatrombopag from days 1 to 180 at 60 mg oral daily and dose adjusted according to platelet count. Two primary endpoints, CR rate and acquired clonal evolution (ACE) at 6 months are monitored at each interim analysis where a go/no-go decision is made by evaluating the posterior probability of the events of interest. The maximum sample size is 50 evaluable patients at the primary 6 months endpoint. Stopping boundaries were calculated with a target false positive rate of 10% under global null recommendations for phase II studies. Trial performance and operating characteristics were assessed with 10,000 simulations. Other endpoints collected until 24 months following enrolment include rate and time to first hematological response, survival, cytogenetic evolution, progression to hematological malignancy, safety and quality of life. Exploratory studies include changes in serum and marrow iron stores, longitudinal cell free DNA somatic mutation analysis and T-cell receptor repertoire studies.

In the NEXT trial, patients with R/R sAA, six or more months following at least one course of horse or rabbit ATG are eligible. All patients receive avatrombopag at 60 mg oral daily from days 1 to 180, with additional IST at the discretion of the treating clinician. Two primary endpoints, hematological response rate and ACE are monitored at each interim analysis with a go/no-go decision as described in the FIRST trial above. Both trials are embedded within the Australian Aplastic Anaemia and Other Bone Marrow Failure Syndromes Registry, which will allow long-term follow-up of participants after completion of the trial.

Results:
The FIRST and NEXT trials have enrolled respectively, 56 and 22 patients. During recruitment, to account for patients' cessation prior to the 6 months primary end-point, the FIRST cohort enrolment number was revised to 58 patients. The combined initial eleven patients commenced avatrombopag at 20 mg daily and increased to a maximum of 60 mg daily over 8 weeks based on hematological response. From the twelfth patient onwards, the starting dose was 60 mg and titrated based on observed tolerability and timing of response rates. Interim analyses have been performed for both trials and stopping boundaries for efficacy or safety have not been met.

Conclusion:
We have successfully recruited 56 of the revised 58 patients for the DiAAMOND-Ava-FIRST target cohort to have 50 patients evaluable for safety and efficacy at the primary time-point of six months. The DiAAMOND-Ava-NEXT study recruitment of 22 patients is ongoing.

Dr Sharon Savage

Genotype – phenotype relationships in the telomere biology disorders

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Aim:
Telomere biology disorders (TBDs), such as dyskeratosis congenita (DC), are a spectrum of illnesses caused by very short telomeres for age due to germline pathogenic variants (PVs) in genes essential in telomere maintenance and encompassing autosomal dominant (AD), autosomal recessive (AR), or X-linked (XLR) inheritance as well as de novo occurrence. This talk will be based on Niewisch et al (PMID: 34852175) which examined the associations between mode of inheritance with phenotypes and long-term clinical outcomes.

Methods:
Two hundred thirty-one individuals with DC/TBDs (144 male, 86.6% known genotype, median age at diagnosis 19.4 years, range 0 to 71.6), enrolled in the National Cancer Institute's Inherited Bone Marrow Failure Syndrome Study, underwent detailed clinical assessments and longitudinal follow-up (median follow-up 5.2 years, range 0-36 to 7]. Patients were grouped by inheritance pattern, considering AD-nonTINF2, AR/XLR, and TINF2 variants separately.

Results:
Severe bone marrow failure, severe liver disease, and gastrointestinal telangiectasias were more prevalent in AR/XLR or TINF2 disease, whereas pulmonary fibrosis developed predominantly in adults with AD disease. After adjusting for age at DC/TBD diagnosis, we observed the highest cancer risk in AR/XLR individuals. At last follow-up, 42% of patients were deceased with a median overall survival of 52.8 years (95% confidence interval [CI] 45.5-57.6) and the hematopoietic cell or solid organ transplant-free median survival was 45.3 years (95% CI 37.4 to 52.1). Significantly better overall survival was present in AD versus AR/XLR/TINF2 disease ($p<0.01$), while patients with AR/XLR and TINF2 disease had similar survival probabilities.

Conclusion:
This long-term study of the clinical manifestations of TBDs creates a foundation for incorporating the mode of inheritance into evidence-based clinical care guidelines, and risk stratification in patients with DC/TBDs.

Professor Tracy Bryan

The role of functional genomics in diagnosis of Telomere Biology Disorders

Bryan, Tracy M.[1], Emerson, Matthew[1], Nelson, Niles[2,3,4], Feurstein, Simone[5], Niaz, Aram[1], Cohen, Scott B.[1], Lucas, Sionne[2], Fairfax, Kirsten[2], Dickinson Joanne[2].
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Aim:
There are at least 15 genes that can give rise to telomere biology disorders (TBDs) when mutated. Patients have variable clinical presentations, and TBDs can be challenging to diagnose clinically. A genomic diagnosis for patients presenting with TBD is vital for optimal treatment. Unfortunately, many variants identified during diagnostic testing are variants of uncertain significance. We therefore aim to establish a robust pipeline of functional analysis of variants in TBD genes, to enable molecular diagnoses for more TBD patients and their families.

Methods:
We have established a pipeline in which patient variants are introduced into plasmid-expressed telomerase genes in HEK293T cells, and telomerase is purified and subjected to biochemical analysis. Telomerase catalytic activity is measured using a quantitative assay, and the ability of the enzyme to assemble its components and interact with DNA is also measured quantitatively. *In vitro* assays are complemented by cellular assays measuring the ability of telomerase to traffic to telomeres and maintain telomere length. We have also systematically reviewed the literature to determine sensitivity and specificity of currently used assays, and to identify the steps required to improve their diagnostic utility[1].

Results:
The number of controls used in our assays has been increased to meet American College of Medical Genetics and Genomics (ACMG) guidelines for application of functional data to variant classification. Functional analysis has been performed on *TERT* gene variants found in 10 Australian patients to date, providing evidence either for or against pathogenicity in each case. This information has been used to guide management, treatment and reproductive decisions in the patients and their extended families. The analyses have also contributed to understanding of telomerase biochemical mechanisms; for example, an unexpected interaction between two different *TERT* variants was revealed[2].

Conclusions:
An improved functional genomics pipeline for patients with variants of unknown significance in TBD genes will greatly increase the number of families receiving a definitive molecular diagnosis, enabling better treatment and management decisions.

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Dr John Mackintosh

Telomere Shortening and Associated Gene Mutations in Adults with Pulmonary Fibrosis: Data from a Tertiary Australian Pulmonary Fibrosis Centre

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Aim:
Mutations in telomere associated genes are increasingly recognised in adults with pulmonary fibrosis (PF), however, genetic testing is rarely performed. The aim of this study was to assess the utility of genetic testing in adults with PF.

Methods:
We performed a retrospective cohort study of adults with PF referred for genetic testing between January 2017 and June 2023 through review of an established local database.

Results:
Forty-three adults (35 male, median age 51 years (range 21-75)) with PF have undergone paired peripheral blood mononuclear cell flow-FISH telomere length (TL) measurement and genetic testing. A positive family history for PF was present in 47%. TL was ≤10th centile in 30/43 (70%) and <1st centile in six of those cases. The frequencies of non-pulmonary features of a telomere biology disorder were: hair greying before the age of 30 years in 35%, mean corpuscular volume ≥97 in 23%, platelet <150 in 14%, neutrophils <2.0 in 12% and lymphocyte count <1.0 in 12%.
A mutation in a telomere associated gene was identified in 14/32 (44%) cases, with 8 in TERT, 4 in PARN, 3 in RTEL1, 1 in TERC, with two cases having mutations in both PARN and TERT. Seven of the mutations were considered likely pathogenic, the remaining nine being variants of uncertain significance. Of the 14 cases with a telomere associated mutation, three had a TL >10th centile, two of which had mutations in RTEL1. There was no family history of PF in 5/14 cases with a gene mutation. Genetic results are pending in 11 cases.

Conclusion:

Telomere shortening and/or mutations in telomere associated genes were frequently identified in selected adults with PF, many of whom did not have a family history of PF or other features of a telomere biology disorder. More work is required to confirm the pathogenicity of more than half the variants discovered.

Dr Ashley Yang

Development of a disease model and gene therapy for telomere-related bone marrow failure

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Aim:
Inherited mutations in telomerase and other telomere-related proteins result in abnormally short ends of chromosomes (telomeres) and lead to a spectrum of diseases known as telomere biology disorders (TBDs). One of their major clinical manifestations, depletion of haematopoietic stem cells, can result in bone marrow failure (BMF), the leading cause of death (~65%) in TBD patients. The only curative treatment, haematopoietic stem cell transplantation, has a low success rate for short-telomere patients and is associated with various complications. However, there is no physiologically relevant animal model available for elucidating disease mechanisms and developing novel therapeutics.

Methods:
In order to establish *in vitro* and *in vivo* models of telomere-related BMF syndromes in clinically relevant cell types, we are optimising CRISPR-based gene-editing strategies through either homology-directed repair (HDR) [1] or homology-independent targeted integration (HITI)[2] in human haematopoietic stem and progenitor cells (HSPCs).

Results:
We successfully introduced a single nucleotide polymorphism (SNP) into the *TERC* gene via an HDR strategy in $CD34^+$ HSPCs. Almost 100% allele editing efficiency was achieved in GFP^+ enriched HSPCs (with 44% precise editing in the pre-enrichment sample). The level of *TERC* expression after gene editing was not significantly different from mock-treated cells, and the processing of this non-coding RNA was not interrupted. We verified that editing of the most primitive HSPC population was achieved, and lineage differentiation of the edited HSPCs was similar to mock-treated cells. No insertions or deletions were detected at the 10 top off-target sites predicted by the program CRISPOR in edited K562 and HSPCs.

Conclusion:
Engraftment of these CRISPR-edited HSPCs into immunodeficient mice will enable the first "humanised" mice model for not only the functional characterisation of novel mutations and fundamental research into TBD mechanisms, but also for the development of a preclinical gene therapy strategy for inherited BMF.

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Associate Professor Rachel Koldej

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

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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.

Methods:
Spatial proteomics 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.

Dr Ashvind Prabahran

Comprehensive Evaluation of Immune Microenvironment in Poor Graft Function following Allogeneic Stem Cell Transplantation

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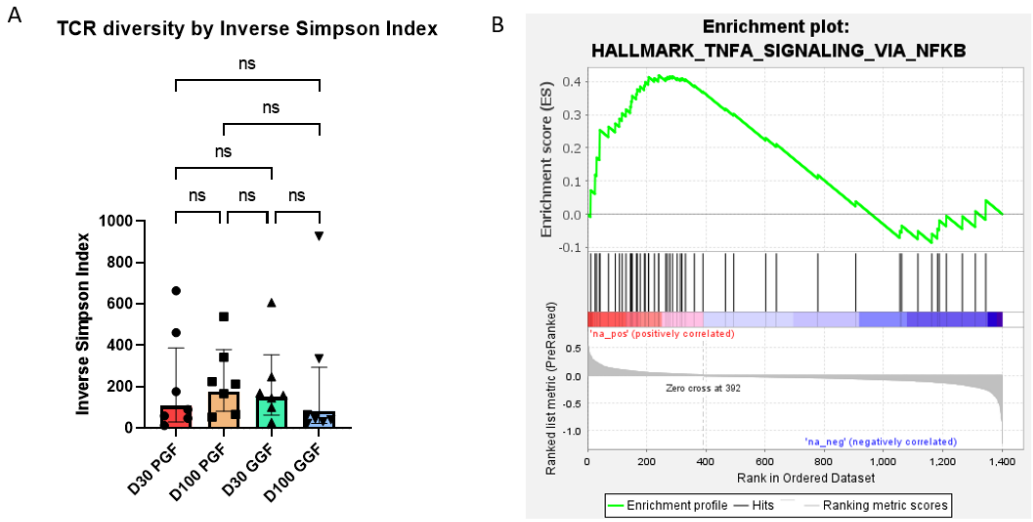
Aim:
1. Poor Graft Function (PGF) defined by multilineage cytopenias in the setting of complete donor chimerism following allogeneic stem cell transplantation (alloSCT) is associated with Graft versus Host Disease (GVHD), non-CMV viral reactivation and ICU admission in 1st 30 days of alloSCT, suggesting an immunologic basis to the syndrome.
2. 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).

Methods:
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.



Associate Professor Amee George

Identification of biomarkers and therapies which modulate the canonical nucleolar surveillance pathway in Diamond Blackfan Anaemia

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Aim:
One of the molecular mechanisms may account for the impaired proliferation and cell death associated with bone marrow failure in Diamond-Blackfan Anaemia (DBA) is the aberrant activation of the nucleolar surveillance pathway (NSP), where disruptions ribosome biogenesis such as those due to ribosomal protein (RP) insufficiency result in the sequestration of the E3 ubiquitin ligase murine double minute 2 (MDM2) by free RPs (predominantly the 60S RPs, L5 and L11) in a complex with 5S rRNA, leading to the accumulation of p53 and subsequent induction of cell cycle arrest or apoptosis. In the case of DBA, the NSP is aberrantly activated, and elevated p53 protein results in preferential apoptosis or cell cycle arrest of the erythroid progenitors required for red blood cell development. Moreover, it has also been proposed that the reduced levels of functional ribosomes in surviving erythroid cells exhibit altered translation of mRNAs that encode proteins critical for erythropoiesis.
To date, our research program has been developed around the central hypothesis that understanding the molecular mechanism(s) by which DBA-causing RP mutations activate p53, leading to the death of erythroid progenitors, would enable us to (i) understand the molecular mechanism underlying the BMF observed in DBA and (ii) enable the identification of novel biomarkers/therapeutic targets for the treatment of patients with DBA, as well as other ribosomopathies for which the NSP is activated.

Methods:
To address this, we have used high content screening technologies to perform genome-wide loss-of-function (RNAi) and gain-of-function (ORF) screens, and FDA-approved compound screens to identify the critical genes and pathways implicated in the p53-mediated NSP due to RPS19 insufficiency (as observed in DBA) and how this response can be therapeutically modulated.

Results:
We have uncovered a suite of novel genes/biological processes involved in the NSP, in particular, the ribosome biogenesis candidate HEATR3, identified in our screen to modulate the p53-dependent NSP. We will also briefly discuss our work identifying clinically approved therapeutics that can ameliorate NSP activation/p53 stabilisation using *in vitro* and *in vivo* approaches as well as combination therapies identified from the screens, which demonstrate a high degree of efficacy in aggressive pre-clinical models of acute myeloid leukaemia.

Conclusion:
Taken together, this information will enable a better understanding of the NSP and therefore the molecular basis of DBA, as well as enable the identification and development of novel treatments and combination therapies for patients with ribosomopathies and cancer.

Dr Stephen Ma

The under-recognized phenotype of germline GATA1 disease in females.

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Aim:
The *GATA1* gene encodes a DNA-binding transcription factor essential to the normal development of hematopoietic cells. Germline *GATA1* variants are classically described as causing an X-linked recessive (XLR) disorder with cytopenias manifesting in varying lineages and are considered rare 'Diamond-Blackfan Anemia-like' diseases. The vast majority of reported cases are male and an increased rate of miscarriages in heterozygous females is thought to occur due to increased fetal death in male hemizygotes. The phenotype of this XLR condition in females is rarely described and poorly understood.

Results:
We have identified 4 female patients with thrombocytopenia and/or anemia attributable to deleterious *GATA1* germline variants. Three *GATA1* variants were previously unreported and one variant has been shown to be *de novo*. All patients had cytopenias demonstrated in childhood/teenage years and represented diagnostic challenges, with two receiving ineffectual therapy for an erroneous diagnosis of immune thrombocytopenia. Marrow morphology in 3 patients was hypercellular with features initially diagnosed as myelodysplastic syndrome. The genetic diagnosis of a germline disorder due to a *GATA1* variant was achieved in these female patients at the ages of 26, 29, 38 and 55 years. We performed X-inactivation studies on one patient which showed evidence of high skewing. Three patients had experienced miscarriage/s (2 with a maternal history of the same) with the fourth patient deemed too high risk for pregnancy.

Conclusion:
In summary, we describe the emerging phenotype of *GATA1* germline disease in females as a result of skewed X-inactivation. This disease is likely under-recognized in females and these patients are at significant risk of inappropriate treatment due to misdiagnosis.

Maddie Riewoldt's Vision.

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