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Centre of Research Excellence in Bone Marrow Biology
presents the:

2025 NATIONAL SYMPOSIUM ON BONE MARROW FAILURE SYNDROMES

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Victorian Comprehensive Cancer Centre

ABSTRACT BOOK

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SPEAKER ABSTRACTS

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Dr Marta Derecka

Transcriptional control of bone marrow niche in normal and pre-malignant hematopoiesis

Lyudmila Tsurkan[1], Melodie Doute[1], Jia-Hua Qu[1], Te Ling[1], Nicholas Morchel[1], Amha Atakilit[2], Bridget Marcellino[3], Ronald Hoffman[3], John Crispino[1], Dean Sheppard[2], Jeremy Crawford[1], Marta Derecka[1]

[1]St. Jude Children's Research Hospital, [2]Department of Medicine, University of California, San Francisco, [3]Tisch Cancer Institute, Division of Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai.

Aim:
Hematopoiesis occurs in bone marrow (BM) microenvironment that includes mesenchymal stromal cells (MSCs). In malignancies, aberrant hematopoietic cells corrupt MSCs, which impairs normal hematopoiesis, but supports the malignant clones. In myelofibrosis (MF), chronically inflamed MSCs produce excessive amount of extracellular matrix (ECM) leading to BM fibrosis and consequently to extramedullary haematopoiesis, hepatosplenomegaly, and anemia. While the genetic events that initiate myelofibrosis in hematopoietic cells are well defined, less is understood about the BM fibrosis. Our major goal is to elucidate the molecular mechanisms that drive fibrotic remodelling of BM niche.

Methods:
Mouse model of MF based on transplantation of hematopoietic progenitors expressing MPL^{W515L} mutation; MSC-specific conditional knockout mouse models; co-cultures of human MSCs with hematopoietic progenitors derived from MF patients.

Results:
We show that transcription factor early B cell factor 1 (EBF1) is a key regulator of MSC function by controlling the expression of ECM, integrins, and proinflammatory cytokines. EBF1 is upregulated in MSCs from MF mouse models and MF patients and EBF1-deficient MSCs are resistant to fibrotic remodelling. Moreover, transplantation of MPL mutant cells into mice with MSC-specific deletion of *Ebf1* results in reduced BM fibrosis and diminished expansion of mutant myeloid cells. We identified ITGB8 as an EBF1-regulated gene in the MSCs, which contributes to BM fibrosis. *Itgb8* deletion renders MSCs resistant to MPL mutant cells, which results in decreased expression of fibrotic markers. Importantly, MF mice treated with ITGB8 neutralizing antibodies exhibit reduced disease burden, decreased marrow fibrosis, reduced frequencies of MPL mutant cells, and lower inflammation in the BM. Also, mice with MSC-specific deletion of ITGB8 show significantly lower BM fibrosis and reduced expansion of MF mutant cells upon transplantation with MPL mutant cells confirming the phenotype observed with ITGB8 inhibition using neutralizing antibodies.

Conclusion: Our data indicate EBF1 and ITGB8 as key mediators of fibrotic remodelling and suggest that targeting EBF1-ITGB8 axis in the MF niche may have therapeutic benefits.

Professor David Ritchie

Is poor graft function allo-immune aplastic anaemia?

Ritchie, David[1]

[1]The Royal Melbourne Hospital

Allogeneic bone marrow transplantation is a common therapy for a range of blood conditions including Bone Marrow Failure Syndromes. The normalisation of bone marrow function with normalisation of blood counts (good graft function or GGF) is achieved in the majority of patients after allogeneic transplant. However, in some patients, despite having evidence of donor cell engraftment, the bone marrow function remains poor and results in low blood counts with associated infection risks and ongoing transfusion burden. This syndrome if known as poor graft function (PGF) and it shares many clinical features with acquired severe aplastic anaemia (aSAA).

Systematic inquiry into PGF has been scant. In studies performed in my laboratory we have undertaken a direct comparison of the pathophysiology within the blood and bone marrow of patients with PGF, GGD and aSAA and found a spectrum of immunological and inflammatory changes with important differences in each. Indeed the immunology of PGF can be recapitulated in commonly used experimental models of aSAA, highlighting the importance if alloreactivity in the loss and suppression on bone marrow stem cells following allogeneic marrow transplantation, which are likely to be different from that occurring in aSAA.

Importantly, the inflammatory signals identified in patients with PGF represent potentially druggable targets to improve marrow function. These drug combinations are currently being explored in the RESELECT portfolio of clinical trials.

Dr Vashe Chandrakanthan

Bioengineered adult human endothelial cell derived haematopoietic stem cells re-establish haematopoiesis in failed bone marrow.

Chandrakanthan. Vashe[1], Thomas. Daniel[1], Gronthos. Stan[2].

[1]Precision Medicine SAHMRI, Adelaide Medical School, Faculty of Health and Medical Sciences, The University of Adelaide, [2]SAHMRI, School of Biomedicine, Faculty of Health and Medical Sciences, The University of Adelaide.

Abstract:

Haematopoietic stem cells (HSCs) have extensive regenerative 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. Further, current gene-editing techniques for human HSCs lose long-term stemness *in vivo* – limiting the application of gene-editing technology for durable cure of inherited bone marrow failure (IBMF). This leads to many patients not gaining timely access to potentially life-saving targeted therapies and even when they do, early-stage clinical trials are often discontinued due to futility. Further, regenerative medicine for IBMF is currently limited by a lack of known soluble regulators that can enhance, renew, or give rise to long-term adult HSCs. When HSCs colonise the bone marrow, how they form niche and maintained their healthy physiology is not clearly understood.
Our initial discovery was that adult mouse heart, lung and aortic endothelial cells can be induced to undergo Endothelium-to-Haematopoietic Transition (EHT) and generate $CD150^+$, $CD45^+$ long-term (LT) transplantable HSCs, responsible for definitive haematopoiesis for the lifetime of the animal (validated over 850+ transplants-Nature Cell Biology 2022). Based on our single cell transcriptomic analysis and understanding HSC-stromal cell niche formation in transplanted animal models, now we have made the startling discovery and developed a protocol that human endothelium (from adult aorta, heart and umbilical cord) can also be induced to generate HSCs using our specific conditions with specific human signalling molecules, established HSC-stromal cell niche and giving rise to long-term human engraftment in bone marrow failed animals (> 6 months). Our novel protocol findings will help to understand the IBMF HSC-stromal cell niche (in humanised animal models) biology and have enormous therapeutic potential for generating patient-specific stem cells and facilitate gene-editing prior to cell therapy towards IBMF.

Dr Raymond Yip

Spatial transcriptomics analysis of bone marrow microenvironment

Raymond K.H. Yip[1,2,3,4$] Jeremy Er[1,3,5,$], Lei Qin[3,6,7], Quoc Hoang Nguyen[3,6,7], Allan Moyter[2,3] ,Joel S. Rimes[1,3], Amanda Light[1,3], Ruvimbo D. Mishi[2], Ling Ling[2], Casey J.A. Anttila[2], Ellen Tsui[2], Daniela Amann-Zalcenstein[2,3], Mark R. Dowling[5,8], Kelly L. Rogers[2,3,*], Rory Bowden[2,3,*], Yunshun Chen[3,6,7,*], Simon J. Harrison[4,8,9*], Edwin D. Hawkins[1,3,4*]

[1]Inflammation Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, 3052, Australia, [2]Advanced Technology and Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, 3052, Australia, [3]Department of Medical Biology, The University of Melbourne, Parkville, VIC, 3010, Australia, [4]Colonial Foundation Diagnostics Centre, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, 3052, Australia, [5]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Parkville, VIC, 3052, Australia, [6]ACRF Cancer Biology and Stem Cells Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, 3052, Australia, [7]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, 3052, Australia, [8]Centre of Excellence for Cellular Immunotherapy, Peter MacCallum Cancer Centre, Parkville, VIC, 3052, Australia, [9]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC, 3052, Australia. [$]These authors contributed equally. [*]These authors jointly supervised this work.

Aim:
The bone marrow microenvironment regulates the differentiation of haematopoietic stem cells, bone metastasis of solid tumours as well as disease progression and therapy resistance in haematological malignancies such as multiple myeloma. We aim to leverage single-cell spatial transcriptomics to dissect the cellular and molecular interactions that support multiple myeloma progression in the bone marrow.

Methods:
We employ a trio of cutting-edge spatial transcriptomics platforms, namely Visium, MERSCOPE and Xenium to study murine and human bone marrow niche in healthy and plasma cell dyscrasias conditions.

Results:
We report the generation of first-of-its-kind spatially resolved transcriptomic maps of the murine and human bone marrow at single cell resolution. We elucidate that malignant plasma cells form spatially segregated microenvironments with distinct transcriptional profiles and cell composition.

Conclusion:
This finding challenges the longstanding dogma that malignant cells form a universal microenvironment to drive disease progression and suggests clonal microenvironment may contribute to the evolution of therapy-resistant clones.

Professor Zoe McQuilten

Efficacy and safety of avatrombopag in addition to immunosuppressive therapy (IST) for patients with previously untreated sAA.

Zoe K. McQuilten[1,2#], Lucy Fox[1,3#], Vanessa Fox[1], Lauren Young[1], Piers Blombery[3,4], Thao Le[1], Ilona Cunningham[5,6], Jennifer Curnow[7], Stephane Heritier[1], Alisa M. Higgins[1], Devendra Hiwase[8], Robin Filshie[9], Frank Firkin[9], Paul Lacaze[1], Kylie Mason[3], Anthony K. Mills[10], Dominic Pepperell[11], Sushrut Patil[12], William Stevenson[6,13], Jeff Szer[3,4], Neil Waters[1], Kate Wilson[6], Stephen B. Ting[1,14*] and Erica M. Wood[1,2*]

[1] Monash University, Melbourne, [2]Monash Health, Haematology Department, Melbourne, [3]Department of Clinical Haematology, Peter MacCallum Cancer Centre & The Royal Melbourne Hospital, Melbourne, [4]University of Melbourne, Melbourne, [5]Concord Repatriation General Hospital, Concord , Sydney, [6]University of Sydney, Sydney, [7]Haematology Department, Westmead Hospital, Sydney, [8]Royal Adelaide Hospital and South Australian Health & Medical Research Institute (SAHMRI), Adelaide, [9]Haematology Department, St Vincent's Hospital, Melbourne, [10]Princess Alexandra Hospital and University of Queensland, Brisbane, [11]Haematology Department, Fiona Stanley

Hospital, Murdoch, [12]Haematology Department, Alfred Hospital, Melbourne, [13]Haematology Department, Royal North Shore Hospital , Sydney, [14]Eastern Health, Department of Haematology, Melbourne, all in Australia.
#Co-first authors; *Co-senior authors

Aim:
The addition of the thrombopoietin-receptor agonist (TPO-A) eltrombopag to immunosuppressive therapy (IST) has been shown to improve responses in severe aplastic anemia (sAA). The aim of this study was to investigate the efficacy and safety of avatrombopag in addition to IST for patients with previously untreated sAA.

Methods:
We conducted an investigator-initiated, single arm Bayesian Optimal Phase II trial (ACTRN12619001042134). We enrolled patients aged 18 years or older with sAA with no prior antithymocyte globulin (ATG)-based IST. Participants received IST (equine ATG and ciclosporin) plus avatrombopag at a maximum dose of 60mg daily orally, dose adjusted according to platelet count, from days 1 to 180. Participants who achieved a partial response (PR) at day 180, defined as transfusion independent with platelet count >20 x 10^9/L and neutrophils >0.5 x 10^9/L, were able to continue avatrombopag for an additional 6 months.

Two co-primary endpoints were assessed at 6 months: 1) complete response [CR], defined as haemoglobin >10 g/dL, neutrophils >1.0 x 10^9/L and platelets >100 x 10^9/L; and 2) acquired clonal evolution [ACE], defined as a new clonal cytogenetic (CG) abnormality or diagnosis of MDS or acute myeloid leukaemia.

Results:
Between October 2019 and August 2023, 56 patients were enrolled from 12 hospitals in Australia, with a median age of 58 years (IQR 40 to 67 years). Of the 56 participants, 7 did not reach evaluation at 6 months due to death (n=3), proceeding to allogeneic stem cell transplant (n=2) and withdrawal for other reason (n=2).

Of the 49 participants with evaluable primary outcome data, CR occurred in 17 (35%) participants (95% credible interval 30% to 46%) and ACE occurred in 2 (4%) participants (95% credible interval 3% to 11%).

Conclusion:
Avatrombopag in addition to IST in treatment-naïve sAA patients resulted in CR of 35%, and ACE of 4% at 6 months. Haematological response was comparable to that reported in the phase III trial of eltrombopag in addition to IST in a similar patient population.

Dr Ashvind Prabahran

Safety and Efficacy of First Line Immunosuppression with ATG and Ciclosporin in Older Patients with Aplastic Anaemia

Ashvind Prabahran*[1,2,3,4], Jibran Durrani*[1], Juan Coelho-Da Silva[1], Ruba Shaloub[1], Jennifer Lotter[1], Olga Rios[1], David Ritchie[2,3,4], Colin O. Wu, Bhavisha A. Patel[1], Neal S. Young[1], Emma M. Groarke[1]

[1]National Heart Lung and Blood Institute, National Institutes of Health, Bethesda MD USA.
[2]Department of Clinical Haematology and Bone Marrow Transplantation, Peter MacCallum Cancer Centre/ The Royal Melbourne Hospital, Melbourne, VIC, Australia.
[3]ACRF Laboratory, The Royal Melbourne Hospital, Parkville, VIC, Australia, [4]Department of Medicine, University of Melbourne , Parkville, VIC Australia.
*Co-first authors; elements of the work have been presented elsewhere previously.

Aim:
There remains uncertainty regarding the safety and tolerability of immunosuppressive therapy with anti-thymocyte globulin and ciclosporin in older patients with aplastic anaemia (AA). We performed a retrospective analysis of a cohort of AA patients treated with immunosuppressive therapy with the following aims:

1. Compare type and frequency of post IST toxicities in an older (≥60 years) versus younger (<60 years of age) cohort of SAA patients

2. Evaluate clinical baseline predictors of toxicity to IST
3. Describe disease outcomes for older patients following IST

Methods:
This was a single-centre, retrospective analysis evaluating data collected as part of two previous prospective studies evaluating the role of immunosuppression in the treatment of newly diagnosed AA.

Results:
Of 245 eligible patients, 54 were older and 191 were younger. Older patients had a similar frequency of SAEs, ICU admissions and hospital length of stay compared to younger patients. Older patients had a higher frequency of cardiac events due to IST, but none resulted in death. Older patients had worse long-term overall survival, more relapse and clonal evolution post IST. However, older patients who responded to IST had a similar survival at median follow up to younger patients.

Conclusion:
Disease related factors and limited therapeutic options in refractory disease likely contribute to poorer outcomes in older patients, not complications of upfront IST. Therefore, IST should be considered 1st line therapy for older SAA patients.

Rachel Edwards

Understanding the Symptom Burden of Paediatric Bone Marrow Transplant: Insights from Patient Reported Outcome Measures

Edwards, Rachel M[1,2,3], Shergold, Jill[1], DeAmbrosis, David[1], Keogh, Samantha[2], Bradford, Natalie[2]

[1]Queensland Children's Hospital, [2]Queensland University of Technology, [3]EviQ Education.

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. Optimal symptom management is dependent upon accurate assessment and appropriate intervention. 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 at point of care to inform clinical management in BMT services for young people.

This research aimed to explore the symptom burden of young people undergoing BMT and establish the feasibility and acceptability of routine use of PROMs at point of care.

Methods:
This health services mixed methods research project explored the symptom burden of young people, aged two to eighteen years. The use of a PROM during hospital admission for BMT was piloted for feasibility and acceptability. Patients recruited to the study were asked to complete the PROM daily during their admission for BMT. Semi-structured interviews were also conducted to determine the symptom experience of young people along with acceptability of PROM collection during BMT.

Results:
Thirty patients who were screened for eligibility, n= 22 (73%) were deemed suitable by the clinical team and of these, n=16 (73%) were recruited, completing 243 PROM data sets.

Physical symptoms causing the most bother included pain (other than headache), changes to appetite, diarrhoea, fatigue, and mouth sores. Over a quarter of the PROM (n=63) also recorded most bother from psychological symptoms such as feeling disappointed/sad, scared/worried, cranky/angry, or changes to body image.

Thirty-nine percent of participants reported that psychological symptoms were not addressed as part of usual care. This contrasts with the other physical and somatic symptoms that were addressed 80.2% – 92.2% of the time.

Conclusion:
Symptom burden in BMT is high and noticeably, there are symptoms that young people experience that are not addressed in routine clinical care. These pilot results warrant further research to determine enablers and barriers for implementation of PROMs at point of care. Interventions to support nursing staff to manage psychological symptoms are needed.

Alice Maier

The Role of Neuropsychology in Haematopoietic Stem Cell Transplant Planning for Bone Marrow Failure Syndromes

Maier, Alice[1,2,3], McCarthy, Maria[1,2,3], De Luca, Cinzia1[1,2,3]

[1]The Royal Children's Hospital, [2]Murdoch Children's Research Institute, [3]University of Melbourne

Aim:
Children with bone marrow failure syndromes (BMFS) undergoing haematopoietic stem cell transplantation (HSCT) often face significant disease and treatment-related challenges. These can impact their cognitive, social, and educational development, with far reaching effects on quality of life. Extensive research exists on managing such challenges for children with malignant conditions undertaking HSCT. However, there is limited understanding and few recommendations for managing these difficulties in children undertaking HSCT for non-malignant conditions.

This study has two primary aims. First, to evaluate the feasibility of implementing a comprehensive neurobehavioral assessment and care plan protocol at the Royal Children's Hospital, Melbourne. Second, to assess the protocol's utility and acceptability from the perspectives of parents, patients, and healthcare providers. A secondary aim is to develop evidence-based recommendations for managing children's neurobehavioral, educational, and family needs before, during, and after HSCT.

Methods:
This is a prospective cohort study examining the feasibility, acceptability, and utility of a neurobehavioral assessment and care plan in children with non-malignant conditions requiring HSCT at the RCH. This study evaluates the implementation of a neurobehavioral assessment and care plan protocol developed at the Royal Children's Hospital (Melbourne). Analysis will be conducted using Slade's Utility Framework. The assessment protocol was developed through a co-design framework with healthcare professionals and parents. It includes pre- and post-transplant assessment of cognitive skills, behaviour and emotional functioning, educational achievement, and family functioning.

Results:
Pending final analysis

Conclusion:
Pending final analysis

Dr Marcin Wlodarski

MDM4 haploinsufficiency leads to P53-mediated bone marrow failure

Richa Sharma[1] , Senthil Velan Bhoopalan[1], Robert Meyer[2], Lei Han[1], Swarna Beesetti[1], Jean Soulier[3], Shondra M. Pruett-Miller[1], Fabian Beier[4,] , Marcin W. Wlodarski[1]

[1]St. Jude Children´s Research Hospital, Memphis, TN, USA.

[2]Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, 52074 Aachen, Germany.

[3]Institut de Recherche Saint-Louis (IRSL), Université Paris Cité, 75010 Paris, France.

[4]Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, RWTH Aachen University, Aachen, Germany.

Background:
Bone marrow failure (BMF) syndromes encompass a heterogenous group of diseases characterized by impaired hematopoiesis and risk of evolution to myelodysplastic syndrome (MDS) and leukemia. We report 6 unrelated individuals with variable BMF phenotypes and hypocellular MDS presenting at a median age of 10 years (4 weeks - 53 years).

Methods/Results:
Unbiased genomic analysis revealed germline heterozygous variants in MDM4, including 4 null (frameshift, nonsense, and splice-site resulting in premature truncation confirmed by RNA sequencing) and 2 missense variants. Mechanistically, MDM4 mutations lead to loss-of-function with enhanced p53 activation. We used CRISPR/Cas9 to delete MDM4 in healthy donor hematopoietic stem and progenitor cells (HSPCs). The resulting MDM4-haploinsufficient HSPCs exhibited increased p53 activity, impaired colony-forming capacity, and reduced engraftment potential in immunodeficient mice. Complementation studies revealed the functional necessity of both p53-binding and RING-finger domains in MDM4-mediated hematopoietic regulation. To study variant effect in a genetic background free of potential confounders, we introduced patient-specific MDM4 variants into induced pluripotent stem cells (iPSCs). MDM4-mutant iPSCs yielded significantly reduced erythroid and myeloid cell populations and exhibited increased p53 activity, as evidenced by elevated p21 expression, confirming the role of MDM4 in regulating hematopoiesis through p53 modulation. Transcriptome analysis of iPSC-derived hematopoietic cells revealed upregulation of p53 pathway. Importantly, one patient acquired TP53 mutations, suggesting maladaptive somatic rescue in the context of MDM4 deficiency.

Conclusion:
Our findings establish MDM4 deficiency as a new “TP53-opathy” BMF syndrome with variable hematopoietic manifestations. This study also highlights the critical role of the MDM4-p53 axis in maintaining hematopoietic homeostasis.

Professor Graham Lieschke

Evaluating new genes and mutations for bone marrow failure disorders

Lieschke, Graham J.[1,2], Mollanoori Shamsi, Hasan[1], Kapitzke, Elias[1], D’Souza, Gail[1], Briones Miranda, Brenda[1], Pazhakh, Vahid[3], Blombery, Piers[1,3].

[1]Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria, Australia, [2]Clinical Haematology, Peter MacCallum Cancer Centre/Royal Melbourne Hospital, Melbourne, Victoria, Australia, [3]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Victoria Australia.

Aim:
Genomic analysis of patients presenting with a spectrum of undiagnosed haematological disorders potentially indicative of inherited bone marrow failure syndromes (IBMFS) often flags candidate disease genes with plausible patterns of inheritance and in silico predictions of deleterious effects on function. However, functional evidence is needed to support the gene/disease pairing. We use zebrafish reverse genetics as a platform to study the requirement of individual candidate disease genes in haematopoiesis, and where possible to precisely model actual patient disease alleles. The hypothesis tested for each candidate gene evaluated is that its function is required for a specific aspect of normal haematopoiesis.

Methods:
New candidate disease genes were reviewed for their suitability for modelling in zebrafish on the basis of homology, expression and literature review. Zebrafish loss-of-function models were generated by gene editing in embryos of reporter strains selected to facilitate the quantitative scoring of different blood cell types.

Results:
Several examples where zebrafish modelling supports an IBMFS disease/gene pairing will be presented.

In a patient with thrombocytopenia, TPM4 lay in a genomic deletion and its loss was one possible cause for the thrombocytopenia based on prior studies linking the gene with macrothrombocytopenia in mice. Zebrafish tpm4a crispant knockdown embyros on a Tg(mpl:EGFP) reporter background have a significantly reduced number of thrombocytes prior to developing a cardiac developmental defect. This supports a TPM4/tpm4a as a thrombocytopenia disease gene.

In a patient with Diamond Blackfan anaemia (DBA), a small deletion in the RPL31 polyadenylation signal (PAS) sequence was found. Zebrafish rpl31 crispants carrying PAS-deletions demonstrate a requirement for rpl31 in normal development. Zebrafish rpl27 crispants with PAS-deletions manifest an early anaemia. These observations highlight PAS sequence variations as an unusual, probably under-recognised cause of gene impairment, and one that is likely generically applicable across multiple DBA genes.

Conclusion:
Zebrafish are a useful model for functionally evaluating IBMFS gene/disease pairings.

Dr Sean Harrop

Heterozygous germline TET2 loss of function variants associated with an ALPS like phenotype

Sean Harrop[1] , Joshua Casan[1,2], Hannah Rose[3] , Michael Sullivan[4,5,6], Sam Mehr[7] , Henry Ngu[8] , Imogen Caldwell[9] , Joseph McKendrick[10], Mary Ann Anderson[1,2,] Nicole Den Elzen[1] , Erin Goode[1] , Lucy C. Fox[1,2], Stephen Lade[1,2,] Piers Blombery[1,2]

[1] Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, [2] Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia, [3] University Hospital, Geelong, Victoria, Australia, [4] Royal Children's Hospital, Melbourne, Victoria, Australia, [5] Murdoch Children's Research Institute, Melbourne, Victoria, Australia, [6] Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia, [7] Department of Allergy/Immunology, Royal Children's Hospital, Melbourne, Victoria, Australia, [8] Department of Hematology, Auckland City Hospital, Auckland, New Zealand, [9] Department of Pathology and Laboratory Medicine, Auckland City Hospital, New Zealand, [10] Eastern Health, Box Hill, Melbourne, Victoria, Australia.

Germline homozygous loss-of-function mutations in TET2 result in significant childhood immunodeficiency that resembles autoimmune lymphoproliferative syndrome and predisposes to lymphoma. The implications of heterozygous variants are less well understood. We describe four patients with heterozygous germline loss-of-function TET2 mutations that presented with B-cell lymphoma on a background of chronic lymphadenopathy and autoimmune features. This expands the association of germline TET2 mutations with lymphoma and an ALPS-like phenotype to the heterozygous state. Assessment for TET2 mutations and germline origin should be considered in the appropriate context as recognition of these variants may have implications on patient care.

Dr Miles Horton and Dr Thomas Lew

Prime editing to correct genetic drivers of haematologic diseases with minimal off-target toxicity and tuneable in vivo selection

Miles B. Horton[1,2], Thomas E. Lew[1-3], Esther Bandala Sanchez[1], Kerry Ramsay[1], Christine A. White[1], Lucy Fox[3], Carrie van der Weyden[3], Charlotte Slade[1,2], Andrew W Roberts[1-3]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Department of Medical Biology, University of Melbourne, Melbourne, Australia, [3]Clinical Haematology, Peter MacCallum Cancer Centre and the Royal Melbourne Hospital, Melbourne, Australia.

Aim:
To evaluate the safety and feasibility of prime editing to correct pathogenic mutations in haematopoietic cells and to introduce mechanisms of in vivo drug selectivity with commonly used drugs.

Methods:
Peripheral blood mononuclear cells (PBMCs) from healthy donors and patients with monogenic haematopoietic disorders were nucleofected with mRNA transcribing prime editing machinery and guide RNAs to correct pathogenic mutations and introduce drug resistance mutations. Next-generation sequencing (NGS) and flow-based assays confirmed restoration of wildtype phenotype and drug resistance was confirmed in vitro.

Results:
Using subcutaneous panniculitis-like T-cell lymphoma (SPTCL) as a model of monogenic haematopoietic disease, we first demonstrated that prime editing efficiently corrected the pathogenic HAVCR2 mutation and restored TIM3 expression across multiple primary patient samples. Next, we introduced targeted edits in FKBP1A, encoding FKBP12—the obligate target of tacrolimus—to disrupt drug binding, enabling the creation of a genetically corrected, drug-selectable T-cell product. State-of-the-art circle-seq methods confirmed minimal off-target genome toxicity, with significantly reduced unintended edits compared to conventional CRISPR-Cas9/HDR. We confirmed that edited T cells retained sensitivity to other immunosuppressants, including cyclosporine. Finally, these edited T cells demonstrated normal engraftment in vivo. Additional data on other genetic haematologic diseases and drug-gene combinations will be presented.

Conclusion:
Prime editing enables precise and efficient correction of pathogenic mutations in haematopoietic cells while allowing for controlled drug-selectable cell therapies, addressing major challenges in gene therapy.

Dr Ashley Yang

Disease modelling and gene therapy for telomere-related bone marrow failure

Ashley Yang, Vivian Lieu, Mackenzie Labine-Romain, Karen L. MacKenzie, Leszek Lisowski, Tracy M. Bryan

Children's Medical Research Institute, Westmead, NSW 2145, Australia

Aim:
Telomere biology disorders (TBDs) arise from inherited mutations in telomerase and associated proteins, resulting in shortened telomeres and a spectrum of severe diseases. A major critical manifestation is the depletion of haematopoietic stem and progenitor cells (HSPCs), leading to bone marrow failure (BMF). While haematopoietic stem cell transplantation is the only curative treatment, its success rate is low for patients with short telomeres and is often associated with complications. Unfortunately, there is currently no physiologically relevant disease model to elucidate disease mechanisms or facilitate the development of new therapies.

Methods:
To address this gap, we employed homology-directed repair (HDR)-dependent CRISPR-based gene-editing techniques[1] to "edit-in" and "correct-out" patient-specific mutations in HSPCs, enabling in vitro and in vivo models of telomere-related BMF.

Results:
Our approach successfully introduced and reverted a pathogenic mutation in the TERC gene, a key component of telomerase. We achieved near-complete editing efficiency, with over 80% of alleles precisely edited even prior to enrichment with a selectable marker. The current strategy effectively edited the most primitive haematopoietic stem cells, with no off-target edits detected. We verified that gene editing did not disrupt processing of the non-coding RNA encoded by the TERC gene. Mutation-bearing HSPCs exhibited impaired growth and colony formation due to reduced telomerase activity and shortened telomere length.

Conclusion:
We established a clinically relevant in vitro TBD model using HSPCs, enabling the functional characterization of novel mutations and exploration of TBD mechanisms. Engrafting these CRISPR-edited HSPCs into immunodeficient mice will create the first "humanized" mouse model, providing a robust platform for developing a preclinical gene therapy strategy for inherited BMF.

[1]Bak RO, Dever DP, Porteus MH. CRISPR/Cas9 genome editing in human hematopoietic stem cells. *Nat. Protoc.* 2018;13:358-376.

Dr Lorna McLeman

Novel gene editing approaches for Fanconi anaemia

McLeman, Lorna M[1,2,3,5] Deans, Andrew[1,5] Glaser, Astrid[1,5] Conyers, Rachel[2,3,4] Elefanty, Andrew[2,4]

[1]St Vincent's Institute of Medical Research, Melbourne, [2]Murdoch Children's Research Institute, Melbourne, [3]Royal Children's Hospital, Children's Cancer Centre, [4]University of Melbourne, Department of Paediatrics
[5]University of Melbourne, Department of Medicine and dentistry and health sciences.

Aim:
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 due to toxic side effects of chemotherapy used in conditioning and accelerates secondary cancer onset. Gene editing is an alternative near future therapy for FA, that would not have these toxicities. Furthermore, autologous gene therapy units are likely to soon be incorporated into the Australian Public Healthcare sector. We aim to show the novel gene editing technology Prime Editing can be used to efficiently correct gene targets in primary induced haematopoietic stem cells (iHSC).

Methods:
In my project I correct underlying mutations using Prime Editing – a CRISPR based cutting edge technology that can revert DNA errors without dsDNA breaks. This system overcomes the potential concerns for off target mutations with viral gene therapy and also has the potential to be used to target almost all genetic mutations. A/Prof Deans' team has shown over 90% precise gene correction, without selection, in experiments with Prime Editing. This unparalleled level of correction highlights the potential of Prime Editing as a therapy for genetic diseases.

Results:
We have demonstrated high levels of editing efficiency using Prime Editing in iHSC's using a targeted assay EGFP BFP. These edited cells maintain their stem cell phenotype. We have also shown successful gene editing using the system in iHSC's at endogenous gene targets. We continue to work on correcting Fanconi anaemia patient mutations in vitro.

Conclusion:
Prime editing can efficiently correct gene targets in patient derived iHSC's and may be a potential future technology for autologous gene therapies for patients with Fanconi anaemia.

Associate Professor Wayne Crismani

New tools to identify cases of Fanconi anaemia

Michael Sharp[1,2], Caitlin Harris[1], Chirantani Mukerjee[1], Stevan Novakovic[1], Elissah Granger[1,2], Roser Pujol[3,4], Jordi Surralles[3,4], Andrew Deans[1,2], Adam Nelson[5,6], Lisa Worgan[7], Wayne Crismani[1,2]

[1] St Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia, [2]The Faculty of Medicine, Dentistry and Health Science, The University of Melbourne, Parkville, Victoria, Australia, [3]St Pau Hospital, Barcelona, Spain, [4]The

Autonomous University of Barcelona, Spain, [5]Sydney Children's Hospital Network, Sydney, NSW, Australia, [6]School of Women's and Children's Health, University of New South Wales Faculty of Medicine, [7]Clinical Genetics Service, Royal Prince Alfred Hospital, Camperdown NSW Australia.

Aim:
Fanconi anaemia is a heritable DNA repair disorder which has a highly variable clinical presentation, however, all individuals with FA share an underlying predisposition to bone marrow failure and specific malignancies. We present here an atypical case of X-linked Fanconi anaemia where we sought to identify the pathogenic variant within this family.

Methods:
This laboratory research project used a suite of molecular biology, cell culture tools, cytogenetics and clinical information to identify the underlying causal variant. Methods include lentiviral-based genetic complementation, chromosome fragility assays, genomic and transcriptomic sequencing, bioinformatics methods and linkage analysis.

Results:
The underlying mutation which causes Fanconi anaemia in this family is likely a single nucleotide deletion in an intron of FANCB which leads to the exonisation of a SINE-Alu element, which would lead to premature termination of translation. The variant went undetected initially due to somatic mosaicism which will be discussed in this presentation.

Conclusion:
This study highlights the importance of considering less common mechanisms, such as SINE-Alu exonisation, in the identification of pathogenic variants in Fanconi anaemia. This work underscores the need for comprehensive genetic testing, including next-generation sequencing and bioinformatics approaches alongside traditional cytogenetics, to detect subtle mutations that may otherwise be missed.

Dr Paula Rio

Hematopoietic Stem Cell Gene Therapy: Overcoming Barriers in Fanconi Anemia Treatment

Paula Río[1,2]

[1]Division of Hematopoietic Innovative Therapies. Centro de Investigaciones Energéticas Medioambientales y Tecnológicas and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIEMAT/CIBERER), Madrid 28040, Spain, [2]Advanced Therapies Unit, Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid 28040, Spain.

Gene therapy for Fanconi anemia (FA) has been a significant challenge for many years, as none of the previous trials were able to demonstrate engraftment of gene-corrected hematopoietic stem cells (HSCs). Our laboratory has focused on providing this therapeutic option to patients through a phase I/II clinical trial (FANCOLEN-1). Mobilized peripheral blood CD34+ cells from nine FA-A patients were transduced with a therapeutic FANCA-encoding lentiviral vector and reinfused without any preconditioning treatment. Long-term follow-up in this clinical trial has shown a progressive increase in genetically and phenotypically corrected lympho-hematopoietic cells in 6 out of 8 evaluable patients. Importantly, 2 of the 3 patients who were infused with at least 240,000 corrected CD34+ cells/kg showed correction of bone marrow failure (BMF) progression. These results paved the way for the development of a global phase II trial sponsored by Rocket Pharmaceuticals, in which higher numbers of corrected CD34+ cells were infused at an earlier stage of the disease. As a result, faster engraftment and progressive increases in vector copy number have been observed so far in 8 of 12 evaluable patients over 15-54 months of follow-up.

The results from LV-mediated gene therapy and the development of new genome editing tools, such as prime editing, motivated us to test the feasibility of this novel editing approach to correct specific mutations in HSCs from FA patients. The PE3 system demonstrated high performance in healthy donor HSPCs both in vitro and in vivo. More recently, purified BM CD34+ cells from FA patients carrying the FANCA c.295C>T mutation were edited by PE. Current data has confirmed that prime editing efficiently reverts the mitomycin C hypersensitivity of FA HSPCs.

Our findings highlight the relevance of LV-mediated gene therapy as a new alternative for correcting BMF in FA-A patients without genotoxic conditioning and demonstrate the potential of prime editing as a future option for specific mutations in different FA genes.

Dr Marcin Wlodarski

Somatic gene rescue in SAMD9 and SAMD9L syndromes: natural gene therapy or sword of Damocles?

Sushree Sangita Sahoo[1], Charnise Goodings-Harris[1], Sara Lewis[1], Marcin W Wlodarski[1]

[1]St. Jude Children's Research Hospital, Memphis, TN, USA.

Aim:
SAMD9/9L syndromes, a group of multisystem disorders caused by gain-of-function mutations in the SAMD9 and SAMD9L genes. The common denominator are the risk for cytopenia typically following infectious triggers, and the development of monosomy 7 as well as other somatic gene rescue events at SAMD9/9L gene locus. The aim of our study was to characterize the mechanisms, cellular origins, and clinical implications of somatic genetic rescue (SGR) in this syndrome.

Methods:
We analyzed data from 243 published patients with SAMD9/9L syndromes along with information from 62 unpublished cases. Somatic events were characterized using conventional cytogenetic analysis, SNP arrays, and deep sequencing. Additionally, we employed single-cell proteogenomic analysis to simultaneously detect single nucleotide variants, copy number variations, and protein expression in 22 samples from 10 patients, allowing us to trace the clonal architecture and lineage-specific distribution of adaptive events.

Results:
SGR occurs in at least two-thirds of patients with SAMD9/9L syndromes through three distinct mechanisms: (1) monosomy 7 with selective loss of the chromosome carrying the mutant allele; (2) uniparental disomy 7q (UPD7q) resulting in duplication of the wild-type allele; and (3) somatic SAMD9/9L mutations in cis with the germline mutation, providing a compensatory effect. Single-cell analysis revealed complex branching clonal architecture in most patients, with independent origin of different rescue mechanisms. Notably, while monosomy 7 was restricted to the myeloid compartment, other SGR events were frequently lineage-specific, with somatic SAMD9/9L mutations and UPD7q found exclusively in T or B lymphocytes in several patients. This lineage restriction explains why many SGR events do not result in complete clinical remission.

Conclusion:
SGR represents a natural compensatory mechanism in SAMD9/9L syndromes that can lead to complex, lineage-specific clonal hematopoiesis with varying clinical outcomes. Our findings highlight the importance of comprehensive genetic surveillance and lineage-specific analysis to help understand genetic mechanisms driving disease remission and progression.

Associate Professor Chris Hahn

ERG deficiency syndrome – a new autosomal dominant bone marrow failure syndrome displaying somatic genetic rescue

Hahn, Christopher N[1,2,3], Zerella, Jiarna R[1,2], Homan, Claire C[1,2,3], Arts Peer[1,2,3], Lin, Xuzhu[4], Spinelli, Sam[1], Venugopal, Parvathy[1,3], Babic, Milena[1,3], Brautigan, Peter J[1,3], Truong, Lynda[4], Arriola-Martinez, Luis[1], Moore, Sarah[3], Hollins, Rachel[3], Parker, Wendy[1,3], Nguyen, Hung[3], Kassahn, Karin[2,3], Branford, Susan[1,2,3], Feurstein, Simone[5], Larcher, Lise [6], De Fontbrune, Flore S[7], Demirdas, Serwet[8], de Munnik, Sonja[9], Poirel, Helene A[10], Brichard, Benedicte[11], Mansour, Sahar[12,13], Gordon, Kristiana[12,14], Wlodarski, Marcin [15,16], Koppayi, Ashwin[17], Dobbins, Sara[12], Mutsaers, Pim[18], Nichols, Kim[19], Oak, Ninad[19], DeMille, Desiree[20], Mao, Rong[20,21], Crawford, Ali[22], McCarrier, Julie[23], Basel, Donald[23], Flores-Daboub, Josue[24], Drazer, Michael[25], Phillips, Kerry[26], Poplawski, Nicola[26], Birdsey, Graeme[27], Pirri, Daniela[27], Ostergaard, Pia[12], Simons, Annet[9], Godley, Lucy A[17], Ross, David M[1,2,28,29], Hiwase, Devendra K[2,28,29], Soulier, Jean[6], Brown, Anna L[1,2,3], Carmichael, Catherine[4,30], Scott, Hamish S[1,2,3,31].

[1]Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, SA, Australia, [2]Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia, [3]Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, SA, Australia, [4]Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, VIC, Australia, [5]Department of Internal Medicine, Section of Hematology, Oncology & Rheumatology, University Hospital Heidelberg, Heidelberg, Germany, [6]Université Paris Cité, Inserm and Hôpital Saint-Louis, APHP, Paris, France, [7]Service d'hématologie-greffe, Hôpital Saint-Louis, Université Paris Cité, Paris, France, [8]Department of Clinical Genetics Erasmus Medical Center Rotterdam The Netherlands, [9]Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands, [10]Sciensano, Rue Juliette Wytsman 14, 1050 Brussels, Belgium, [11]Department of Pediatric hematology and oncology, Cliniques universitaires Saint-Luc, Brussels, Belgium, [12]Cardiovascular and Genomics Research Institute, St George's University of London, London, United Kingdom, [13]South West Thames Regional Centre for Genomics, St. George's Universities Hospitals NHS Foundation Trust, London, United Kingdom, [14]Dermatology & Lymphovascular Medicine, St. George's Universities NHS Foundation Trust, London, United Kingdom, [15]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [16]Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN, [17]Robert H. Lurie Comprehensive Cancer Center and Northwestern University, [18]Department of Hematology, Erasmus Medical Centre Rotterdam, Rotterdam, The Netherlands, [19]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA, [20]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, USA, [21]Department of Pathology, University of Utah, Salt Lake City, UT, USA, [22]Illumina, Inc., San Diego, CA, USA, [23]Department of Pediatrics, Medical College of Wisconsin, Milwaukee WI, USA, [24]Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA, [25]Section of Hematology/Oncology, Department of Medicine, The University of Chicago, IL, USA, [26]Adult Genetics Unit, Royal Adelaide Hospital, Adelaide, SA, Australia, [27]National Heart and Lung Institute, Imperial College London, London, United Kingdom, [28]South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia, [29]Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, SA, Australia, [30]Department of Molecular and Translational Medicine, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, 3168, [31]ACRF Genomics Facility, Centre for Cancer Biology, Adelaide, SA, Australia.

Aim:
The Australian Familial Haematological Conditions Study (AFHCS) recruits families from Australia and New Zealand with apparent predisposition to bone marrow failure (BMF) and/or haematological malignancy (HM). Comprehensive genomic analyses are performed to identify the underlying genetic cause. We have reported discovery of families with GATA2 and PAX5 variants and also identified variants and expanded phenotypes in genes such as RUNX1, CEBPA, SAMD9L, RPS26, MECOM, BRCA1/2, PALB2 and CHEK2. However, there remain families for which a genetic diagnosis remains to be found.

Methods:
We have performed whole exome or genome sequencing and SNP-arrays on blood, bone marrow or hair samples on over 250 families and used a range of bioinformatic analyses to search for rare likely causal genetic variants.

Results:
A pathogenic germline ERG missense variant was identified in a small Australian family with thrombocytopenia, neutropenia and AML. Subsequently, we identified other missense and premature termination ERG variants in unrelated individuals with cytopenia(s) and predominantly myeloid malignancies, including 2 confirmed de novo variants. Functional studies showed that pathogenic missense variants reside predominantly within the highly conserved ETS domain and are loss-of-function (LOF) as were truncating variants. Interestingly, copy neutral loss-of-heterozygosity (cnLOH) was seen in 5 cases across the ERG locus revealing spontaneous somatic genetic rescue. Of the haematopoietic transcription factors that predispose to BMF/HM, ERG is the first to display somatic genetic

rescue. Such genetic rescue is similar to several other BMF/HM predisposition genes including some FANC and ribosomopathy genes and SAMD9/SAMD9L.

Conclusion:
ERG is a member of a highly complex and well-coordinated network of transcription factors that control haematopoietic stem and progenitor cell production and differentiation into mature blood cells. We have identified ERG as a new germline predisposition gene to BMF and HM, and it displays somatic genetic rescue. Implications of somatic genetic rescue include: 1) milder symptoms, increased age of onset, asymptomatic carriers, 2) difficulty in diagnosis due to <50% variant allele frequency in blood or bone marrow samples, and 3) potential for gene or cell therapy as a preventative strategy against haematopoietic-associated diseases such as BMF/HM.

Dr Parvathy Venugopal

Somatic Genetic Rescue in Bone Marrow Failure – Implications and Opportunities

Venugopal, Parvathy[1,2,3], Zerella, Jiarna[1,2], Lawrence, David[1,2], Hiwase, Devendra[2,4,5], Homan, Claire[1,2,3], Phillips, Kerry[6], Poplawski, Nichola[6], Brown, Anna [1,2,3], Kok, Chung[1,2,3,7], Hahn, Christopher[1,2,3], Scott, Hamish [1,2,3,7]

[1]Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, SA, Australia, [2]Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia, [3]Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, SA, Australia,[4]South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia, [5]Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, SA, Australia, [6]Adult Genetics Unit, Royal Adelaide Hospital, Adelaide, SA, Australia, [7]ACRF Genomics Facility, Centre for Cancer Biology, Adelaide, SA, Australia.

Somatic Genetic Rescue (SGR) is a now well-documented phenomenon across several inherited bone marrow failure (BMF) disorders often leading to milder clinical symptoms. It is hypothesised that clonal evolution driven by inflammatory or infectious processes on the background of pre-existing BMF results in demand-adapted haematopoiesis which can lead to either malignancy development through the acquisition of deleterious variants or spontaneous resolution of symptoms through SGR. The presence of SGR leads to reduced variant allele frequencies in blood making it especially challenging to identify causative germline variants in the absence of true germline tissue such as skin.

Mechanisms of SGR (for e.g. cnLOH, original site reversion variants, second site reversion variants, splice altering reversion variants etc.) are largely determined by the mode of disease inheritance and the type of germline mutation (e.g. single nucleotide variant, small or large indel, frameshift, truncation, splice site). Here we compare and contrast various technological approaches (e.g. long-read sequencing, sc-DNAseq, sc-long-read sequencing, SNP arrays, WGS, FISH) that can be used for detection of different types of SGR and discuss the challenges/limitations associated with them and illustrated with examples where possible.

SGR should be considered when choosing a tissue source for diagnosis in carriers with a mild phenotype or in remission, as well as when using patient-derived cell lines for drug screening involving multiple rounds of cell replication. We are currently focused on experimental methods to mimic or enhance SGR in inherited BMF cell and animal models, and evaluation of the biological impact of such reversion events. There are likely different thresholds of genetic correction required for correction of the different phenotypes and these may vary for different BMF syndromes. Determination of these thresholds can have significant implications on required efficiency of gene manipulations, as well as the manufacturing and quality control processes for autologous cellular therapies.

Dr Lucy Fox

Donor-derived somatic genetic rescue after allogeneic stem cell transplantation from a SAMD9-affected sibling donor

Michelle Tan[1], Nicole Den Elzen[1], Erin Goode[1], Ashvind Prabharan[2], Piers Blombery[1,3,4,5], Lucy C. Fox[1,3,4,5]

[1]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Victoria; [2]Austin Health, Melbourne, Victoria; [3]Clinical Haematology, Peter MacCallum Cancer Centre/Royal Melbourne Hospital, Melbourne, Victoria; [4]Sir Peter MacCallum Department of Oncology, University of Melbourne, Victoria, Australia [5]Transfusion Research Unit, School of Public Health & Preventive Medicine, Monash University, Melbourne, Victoria.

Constitutional heterozygous variants in SAMD9 (sterile alpha motif domain containing 9) and paralog gene SAMD9L (SAMD9-like) are increasingly recognised to cause autosomal dominant inherited bone marrow failure (BMF) syndromes. The clinical spectrum of germline SAMD9 includes multisystem MIRAGE syndrome (MIM #617053), monosomy 7 myelodysplasia (MDS)/acute myeloid leukaemia (AML) (MIM #619041) and BMF. As in other inherited BMF syndromes, SAMD9-associated marrow failure and MDS/AML can be successfully treated with an allogeneic stem cell transplant (alloSCT), with cautious donor selection if considering blood relatives. Here we describe a kindred with a novel germline SAMD9 variant demonstrating variable penetrance and a history of alloSCT three decades earlier from an unrecognised genetically affected sibling donor.

Associate Professor Piers Blombery

Whole genome sequencing for diagnosis in bone marrow failure – the IBMDx study

Blombery, Piers[1]

[1]Peter MacCallum Cancer Centre, Melbourne, Australia

Patients with inherited bone marrow diseases often have a delay in diagnosis which impacts their care. Part of this delay may relate to the available diagnostic tools to establish diagnoses in this setting. Since March 2022, we have been conducting a study to investigate the effectiveness of whole genome sequencing in patients with inherited bone marrow diseases (the IBMDx study). To date we have recruited over 200 patients from around Australia. This presentation will cover the rationale for the study, the diverse data being collected including health implementation and health economic endpoints as well as presenting preliminary genomic data.

Dr Vahid Pazhakh

Pursuing diagnosis after whole genome sequencing: Insights from the IBMDx study

Pazhakh, Vahid[1], Fox, Lucy[1], Den Elzen, Nicole[1], Goode, Erin[1], Roesley, Ain[2], Bell, Katrina[2], Blombery, Piers[1]

[1]Department of Pathology,Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Melbourne, Australia.

Aim:
The aim of the IBMDx study is to explore the potential of whole genome and transcriptomic sequencing (WGS/WTS) in identifying genetic mutations responsible for inherited bone marrow failure syndromes (IBMFS) in Australian patients. This presentation highlights three intriguing cases from the IBMDx study.

Methods:
Eyebrow hair and blood samples from patients with suspected IBMFS were collected for DNA and RNA extraction, respectively. Additionally, blood DNA was extracted for somatic mutation analysis. WGS and WTS data were analysed together to cross-validate the functional effects of candidate variants identified through WGS.

Results:
In the first case, WGS revealed a single pathogenic variant in the HAX1 gene in a patient with congenital neutropenia. Further investigation uncovered a retrotransposon insertion in HAX1 intron 1, confirmed by RNA-seq to cause exon 2

skipping. The second case involved a patient with a Diamond Blackfan Anaemia (DBA) presentation. WGS revealed no striking mutations in known DBA genes; however, RNA-seq showed an unexpected 3'UTR extension in RPL31, subsequently confirmed to be caused by a 2bp deletion in the polyadenylation signal (PAS), which was confirmed as a de novo variant in trio analysis, explaining the patient's phenotype. The third case presented a suspected germline SAMD9 mutation. Somatic testing in this patient revealed somatic reversion mutations in SAMD9, supporting the pathogenicity of a germline gain-of-function SAMD9 mutation.

Conclusion:
These cases demonstrate the power of WGS and WTS in identifying uncommon and complex genetic alterations contributing to rare genetic disorders such as IBMFS. The results emphasise the importance of considering unconventional genetic changes, such as retrotransposons and PAS variants, in the diagnosis of these syndromes. This study highlights how advanced genomic techniques can enhance molecular genetic diagnosis and improve our understanding of the pathogenesis of these rare conditions, ultimately leading to better patient management and care.

Associate Professor Tina Carter

A review of paediatric and adolescent patients diagnosed with Inherited Bone Marrow Failure Syndromes (IBMFS) in Western Australia at Perth Children's Hospital.

Carter, Tina[1]
[1]Perth Children's Hospital, Western Australia

Inherited Bone Marrow Failure Syndromes (IBMFS) are a group of conditions associated with an increased risk of developing aplastic anaemia and a propensity to developing malignancies, particularly acute myeloid leukaemia. These congenital syndromes are typically associated with abnormal physical features making them amenable to prompt diagnosis. However, they can be very subtle or even absent resulting in delayed diagnosis if not considered.

This diagnosis can also be associated with ongoing physical and developmental issues. They can also be severe, sadly incurable and result in early death. The demographics of our population has changed in Western Australia (WA) over the last decade and this diagnosis can be even more of a challenge where there are different languages and cultural values.

Perth Children's Hospital is the only tertiary paediatric hospital in WA. The hospital opened in June 2018. It is the only WA referral centre for children or teenagers with a possible inherited bone marrow failure syndrome or severe aplastic anaemia.

Our isolation has always encouraged collaboration both nationally and internationally and our unit has membership of many paediatric oncology, haematology and transplant groups. Our patients are enrolled on the Australian Aplastic Anaemia and Other Bone Marrow Failure Syndrome Registry (AAR).

Our diagnostic approach to these patients will be discussed. The increase in population, the change in demographics of our population and our ability to diagnose these rare conditions using molecular panels has meant that we have seen an increase in the diagnosis of paediatric and adolescent patients with IBMFS in our state.

A review of some of these cases will be presented with regard to interesting, rare cases, challenges and our continued collaboration with Maddie Riewoldt's Vision and the Peter MacCallum Cancer Centre.

Emma Murphy

Sensitive genomic characterisation of the clonal haematopoietic landscape in individuals with germline GATA2 haploinsufficiency

Murphy, Emma[1]
[1] Wilson Centre for Blood Cancer Genomics, Peter MacCallum Cancer Centre.

Aim:
GATA2 haploinsufficiency syndrome is a germline disease which predisposes individuals to myeloid malignancy. The pre-malignant clonal haematopoietic (CH) landscape of individuals with GATA2 haploinsufficiency has not been extensively investigated to date. We aimed to characterise the clonal haematopoietic landscape of patients with GATA2 haploinsufficiency using ultra-sensitive molecular methodologies.

Method:
Thirteen individuals with GATA2 haploinsufficiency were assessed using an 80-gene haematological malignancy UMI-corrected NGS panel (sensitivity 0.5% VAF). To further explore somatic variants at an ultra-sensitive level, a subset of the cohort underwent Twinstrand Duplex sequencing to detect variants at very low frequency (<0.01% VAF).

Results:
CH was detectable in 9/13 patients by UMI-NGS, including in both the presence and absence of myeloid malignancy. STAG2 was the most frequently mutated gene with variants observed in 6/13 patients (38%) including those without overt haematological malignancy. Sensitive variant detection identified multiple STAG2 variants occurring together (range 1-10 per pt) consistent with oligoclonal clonal haematopoiesis. In one patient with longitudinal sampling, a single STAG2 clone was observed to emerge from an oligoclonal STAG2 mutated background. Notably, 3/13 patients harboured non-canonical MPL exon 12 variants. Finally, ultra-sensitive duplex sequencing and analysis using Twinstrand Duplex AML-29 App on the DNAnexus platform revealed marked further complexity, including multiple previously unrecognised STAG2 variants as well as variants in DNMT3A, ASXL1 and TET2.

Conclusion:
We have described the clonal haematopoietic landscape of GATA2 haploinsufficiency at a sensitive level revealing (i) STAG2 as a dominant early molecular lesion (ii) an oligoclonal model of CH presence (iii) enrichment of previously unrecognised MPL exon 12 variants and finally (iv) a high degree of clonal complexity at ultra-sensitive levels. Increasing understanding of the genomic landscape in GATA2 haploinsufficiency provides greater insights into disease progression and may ultimately inform clinical management.

Dr Simon Wu

The genomic profiles of patients with UBA1 variants across haematological malignancies

Wu, Simon[1,2], Li, Jamilla[3], Kankanige, Yamuna[1,4], Hunter, Sally[1], Thompson, Ella[1,2], Tiong, Ing Soo[1,2,5], Blombery, Piers[1,2,5]

[1]Department of Pathology, Peter MacCallum Cancer Centre, Victoria, Australia, [2]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Victoria, Australia, [3]Department of Pathology, Queen Mary Hospital, Hong Kong, [4]Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Victoria, Australia, [5]Department of Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Victoria, Australia.

Aim:
UBA1 variants, typically Met41 variants, are implicated in the pathogenesis of VEXAS syndrome. Potential causative variants have been identified in other domains of UBA1 in VEXAS syndrome, however, the spectrum across haematological malignancy is relatively unknown. We describe the unbiased assessment of UBA1 across a large cohort of patients with haematological malignancy.

Methods:
All UBA1 coding exons by unique molecular index-corrected NGS, along with 79 other genes recurrently mutated in haematological malignancy, were assessed in consecutive samples referred for diagnostic testing over a 12-month period and correlated with clinicopathological features.

Results:
UBA1 variants were detected in 55/7991 patients (0.7%), at variant allele frequency 2-95% (median 51%), including 5 females. Recurrent variants included Met41 variants (n=34), Ser621Cys (n=4), Ala478Ser (n=3) and Lys746Glu (n=2). Samples with UBA1 variants included bone marrow aspirate (n=42) and peripheral blood (n=13). Correlative bone marrow morphology described vacuolated precursors in 23/42 samples available for assessment, 22 in samples with Met41 variants and 1 sample Ala478Ser.

UBA1 variants were detected in 12/112 patients with suspected VEXAS syndrome, including 11 Met41 variants. 32 samples from patients with myelodysplastic or myelodysplastic/myeloproliferative neoplasms harboured UBA1 variants, including 13 Met41 variants in patients with concurrent VEXAS syndrome symptoms. Other primary indications with UBA1 variants included myeloproliferative neoplasms (4 non-Met41 variants), acute myeloid leukaemia (3 non-Met41 variants), lymphoproliferative disorders (2 non-Met41 variants) and investigation of cytopenias (1 Met41 and 1 non-Met41 variant). Co-mutations in other genes were detected in 37 patients, most frequently in TET2 (n=14), DNMT3A (n=11) and SRSF2 (n=6).

Conclusion:
UBA1 Met41 variants are most typically observed in VEXAS syndrome, however non-Met41 UBA1 variants may be observed in other haematological malignancies. UBA1 variants co-occur with mutations in genes associated with clonal haematopoiesis and myeloid neoplasms. Further study is needed to understand the pathogenicity and clonal evolution of UBA1-mutated haematological malignancies.