AGCTS

AUSTRALASIAN GENE AND CELL THERAPY SOCIETY

14th Biennial Meeting

11th to 13th November 2026

Melbourne Connect, Carlton



On behalf of the Australasian Gene and Cell Therapy Society (AGCTS), it is our great pleasure to invite you to the 14th Biennial Scientific Meeting, Wednesday 11th - Friday 13th November 2026 at the award-winning Melbourne Connect, Carlton.

This is the leading regional forum for gene and cell therapy, bringing together researchers, clinicians, industry partners, and policymakers.

This Conference will highlight how gene and related therapies can be used to develop novel treatments that are rapidly advancing into the clinic. The conference will address advances in gene therapies, spanning work from vector development, genome editing, RNA therapeutics to clinical trials.

We warmly welcome you to Melbourne.

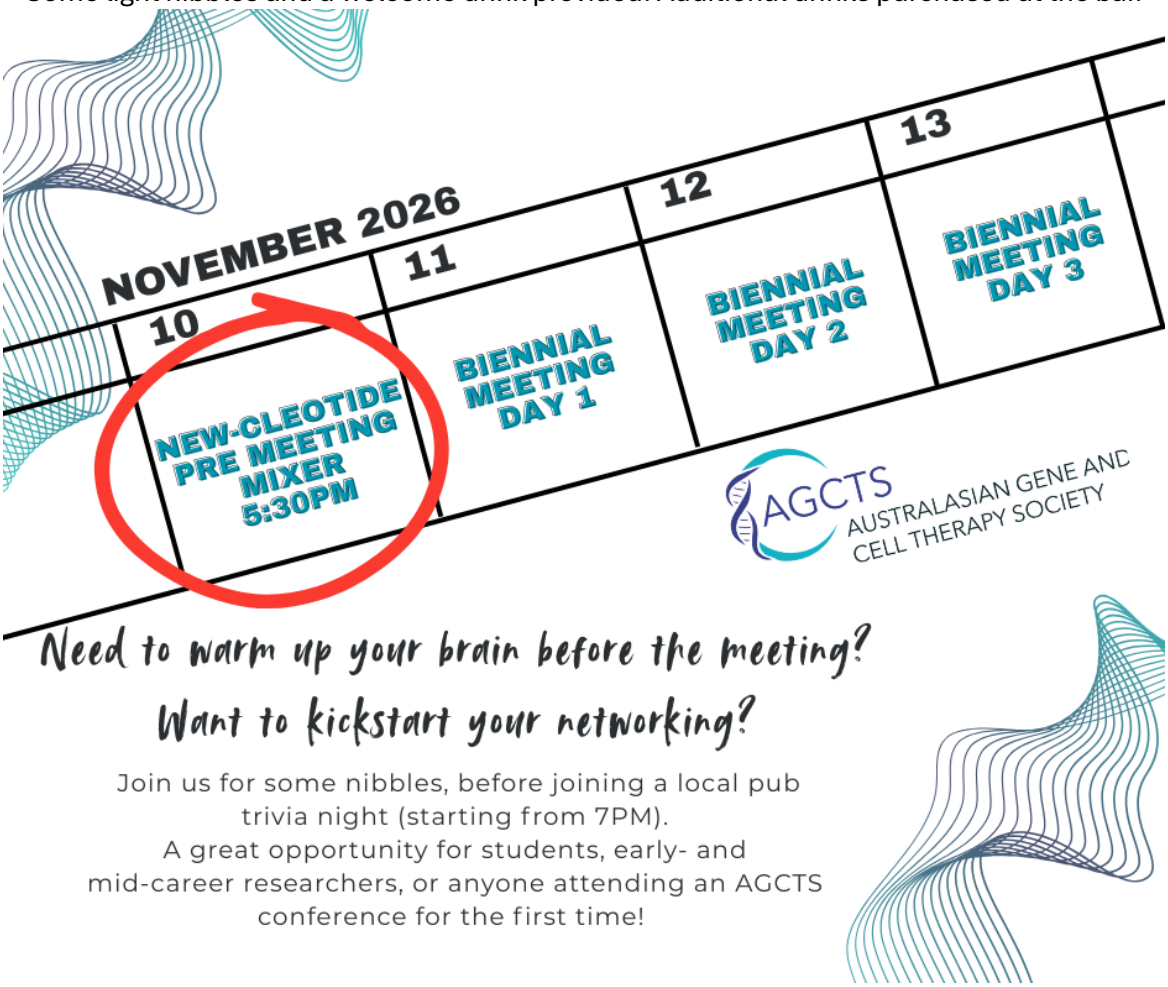


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A. Program overview

Tuesday Evening 10th November 2026
New-cleotide Mixer: 17:30 mixer, 19:00 quiz Prince Alfred Hotel, 191 Grattan Street, Carlton 3053 Social pre-conference event Some light nibbles and a welcome drink provided. Additional drinks purchased at the bar.



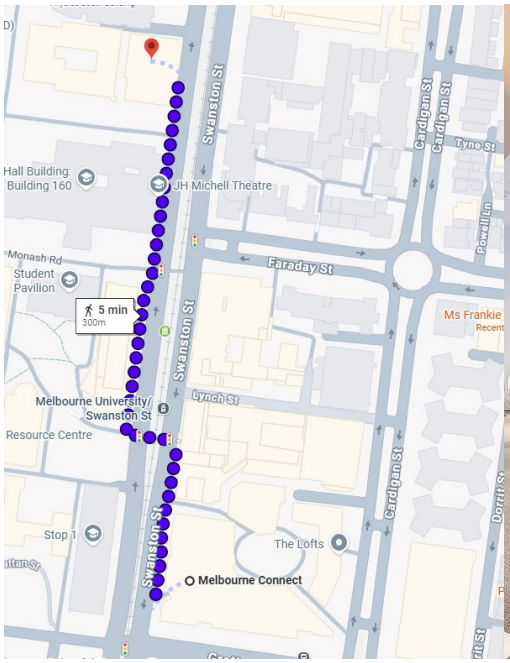
Need to warm up your brain before the meeting?

Want to kickstart your networking?

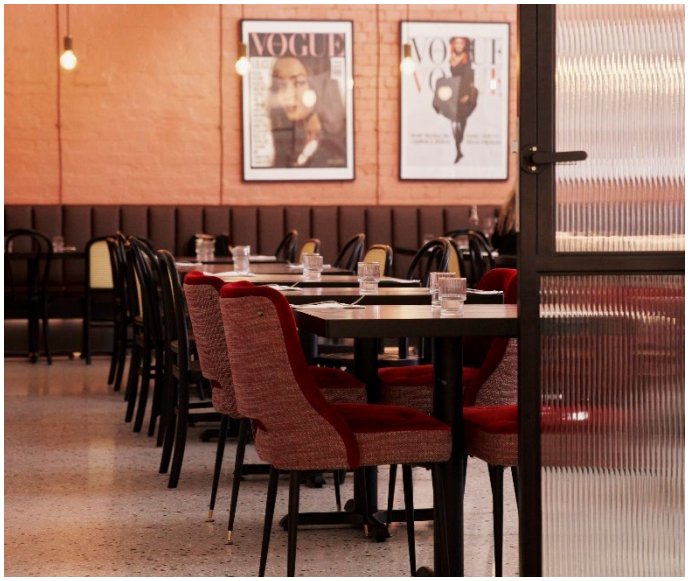
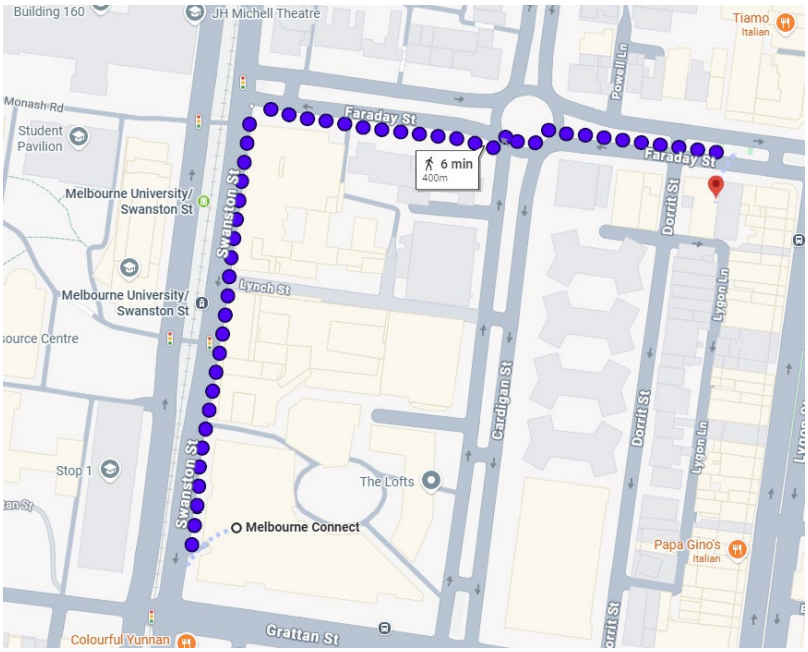
Join us for some nibbles, before joining a local pub trivia night (starting from 7PM).
A great opportunity for students, early- and mid-career researchers, or anyone attending an AGCTS conference for the first time!

Wednesday 11th November 2026		
	8.00	Registration desk opens
	8:45-9:00	President's Welcome and Acknowledgement of Country
Opening Keynote Proudly sponsored by: VectorBuilder Chair: Prof. Leszek Lisowski	9:00-9:45	Invited Speaker – Prof. Krystof Bankiewicz *Clinical advances in Gene Therapy for Neurodegenerative disorders*
Panos Ioannou New Investigator Award Session Part 1 Chairs: A/Prof. Samantha Ginn and Dr. Alexandra O'Donohue	9:45-10:00	Mr. Aidan Murphy *Therapeutic Screening of DYRK1A Targeting Antisense Oligonucleotides in Human Neuronal Model for Alzheimer's Disease*
	10:00-10:15	Dr. Alexander Wykes *Splicing together Rett syndrome: A multi-isoform, single-AAV vector gene therapy approach*
	10:15-10:30	Dr. Elizabeth Kalotay *AAV-mediated DARS1 gene supplementation for hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL): preclinical proof-of-concept in novel mouse models*
Morning tea	10:30-11:00	
Panos Ioannou New Investigator Award Session Part 2 Chairs: A/Prof. Samantha Ginn and Dr. Alexandra O'Donohue	11:00-11:15	Ms. Emily Ronning *Crystallising Gene Therapy for Primary Hyperoxaluria Type 1*
	11:15-11:30	Ms. Eva van Dijk *Challenges to durable genome editing in a rapidly renewing tissue*
	11:30-11:45	Ms. Hsien-Yin Kao *Rewriting NF: Precision Correction of Nonsense Mutation Using Base Editing and Prime Editing*
	11:45-12:00	Dr. Natsuki Sasaki *Discovery of naturally selected human cardiotropic adeno-associated virus to overcome translational barriers for the use in cardiac gene therapy*
	12:00-12:15	Ms. Isabella Trew *Knocking down integrin alpha-4 expression: RNase H-mediated versus splice modulating*
Group Photo	12:15-12:30	
Lunch and Poster viewing	12:30-13:30	
	12:45-13:30	Manned even-numbered poster viewing
Session co-hosted by: AGENt Australia Gene Editing Network Chairs: Dr. Fatwa Adikusuma and Mr Aidan Murphy	13:30-14:15	Invited Speaker -Prof. Kate Quinlan *Genome- and epigenome-editing strategies to upregulate beta-globin compensatory genes*
	14:15-15:00	Invited Speaker – Dr. Adrian Veres *AI-powered sequence design to solve AAV delivery and empower patients with genetic agency*
Afternoon tea	15:00-15:30	
Rare diseases and the patient perspective	15:30-15:45	Prof. Leszek Lisowski and Dr Livia Carvalho
	15:45-16:00	Ms. Emma Bonser
	16:00-16:15	Mr. Steve Hille
	16:15-16:30	Prof. Robyn Langham AM

Chair: Prof. Leszek Lisowski and Dr. Alexander Wykes	16:30-17:00	Panel Discussion
	17:00	End at Melbourne Connect

Wednesday Evening 11th November 2026	
17:15	Welcome reception proudly sponsored by DynoTx Potter Museum of Art, Potter Museum Foyer. The University of Melbourne, 815 Swanston Street (corner of Masson Road), Parkville, Victoria 3010 5-minute walk from conference venue  
19:15	End of welcome reception

Thursday 12th November 2026		
	8:00	Registration desk opens
Genome Editing and Modulation Chair: Prof. Paul Gregorevic and Dr. Elizabeth Kalotay Proudly sponsored by: 	8:45-9:30	Invited Speaker – A/Prof. Gavin Knott
	9:30-9:45	Abstract talk 1 – Dr. Ashley Yang *CRISPR editing as a strategy for gene therapy for telomere-related bone marrow failure*
	9:45-10:00	Abstract talk 2 – Dr. Mawj Adam Mandwie *In vivo editing of the human inactive X chromosome: therapeutic opportunities and epigenomic consequences of AAV-mediated genome engineering*
	10:00-10:15	Abstract talk 3 – A/Prof. Aaron Schindeler *Allele-specific disruption of a patient COL1A1 stop-loss (readthrough) variant that introduces a cryptic SaCa9 PAM site*
Morning tea	10:15-10:45	
AAV Development and Ocular Gene Therapies Proudly sponsored by:  Chairs: Dr. Livia Carvalho and Ms. Emily Ronning	10:45-11:30	Invited Speaker – Dr. Jiang-Hui (Sloan) Wang *Seeing the Whole Retina: A Cross-Species, Animal-Free AAV Capsid for Intravitreal Gene Therapy*
	11:30-11:45	Abstract talk 4 – Dr. Livia Carvalho *Pre-clinical validation of AAV gene therapy for KCNV2 retinopathy improves visual function*
	11:45-12:00	Abstract talk 5 – A/Prof. Wei Deng *Light-guided spatial gene knockout for wAMD treatment*
	12:00-12:15	Abstract talk 6 – Mr. Vasily Medvedev *Australia's First 50 L Recombinant Lentiviral Vector Manufacturing Platform: Development, Scale-Up and Orthogonal Analytical Strategy*
AGM	12:15-13:00	
Lunch and Poster viewing	13:00-14:00	
	13:15-14:00	Manned Odd-numbered poster viewing
Cancer and Gene Therapies Chairs: A/Prof. Chandana Herath and Ms. Hsien-Yin Kao	14:00-14:45	Invited Speaker - Prof. Joy Ho *CAR-T therapy in myeloma: a new era*
	14:45-15:00	Abstract talk 7 – Dr. Jim Vadolas *Therapeutic Silencing of TMPRSS6 Attenuates Iron-Driven Inflammasome Activation and Delays Disease Progression in MDS mice*
	15:00-15:15	Abstract talk 8 – Mrs Muditha Wijesinghe *Flipping the RAS switch: The effect of overexpressing angiotensin-converting enzyme 2 (ACE2) in primary and secondary liver cancer mouse models*
	15:15-15:45	Afternoon tea
Advances and Innovations in Gene Therapy Chairs: Prof. Ian Alexander and Ms. Eva van Dijk	15:45-16:30	Invited Speaker – Ms. Sheila Mikhail
	16:30-17:15	Invited Speaker - Prof. R. Jude Samulski *50 Years of AAV Gene Therapy: A personal perspective*
	17:15	End at Melbourne Connect

Thursday Evening 12th November 2026	
18:30pm	Conference Dinner Ms Frankie 221-229 Faraday Street, Carlton VIC 3053 18:30 arrival drinks and canapés 19:00 Sit down for meal  6-minute walk from conference venue 
21:30	End of Conference Dinner

Friday 13th November 2026		
Alternative Gene Modification Methods Chairs: Prof Ann Simpson and Ms. Isabella Trew	9:00-9:45	Invited Speaker – Dr. Christina Cortez-Jugo *Novel Nanoparticle Systems for the Delivery of Nucleic Acid Therapeutics*
	9:45-10:00	Abstract talk 9 – Dr. Kelly Martinovich *Developing a Small Animal Model to Use as a Proof of Concept for Therapeutic Splice Modulation of LDLR by Antisense Oligonucleotides*
	10:00-10:15	Abstract talk 10 – Dr. Hilary Sheppard *Optimised high rates of gene editing for skin engineering applications using electroporation or a more gentle, yet efficient, cell penetrating peptide*
	10:15-10:30	Abstract talk 11 – Ms Vivienne Kaiser *CRISPR-HITI gene therapy for Usher Syndrome Type 1B in patient-derived inner ear and retinal organoids*
Morning tea	10:30-11:00	
Gene Therapies for Neurological Disorders Proudly sponsored by: tfbs Chairs: Prof. Paul Gregorevic and Dr. Natsuki Sasaki	11:00-11:45	Invited Speaker - Prof. Damjan Osredkar *Towards disease-modifying treatment of CTNNB1 syndrome: from gene to gene therapy*
	11:45-12:00	Abstract talk 12 – Dr. Alexandra O'Donohue *A Change of Base: CRISPR base editing to correct NF2-SWN point mutations*
	12:00-12:15	Abstract talk 13 – Dr. Adam Hagg *A comparative analysis of engineered AAV serotypes to transduce the murine motor unit*
	12:15-12:30	Abstract talk 14 – Dr. Rabail Khan *Towards the development of a safe and effective gene therapy for SLC6A1*
Lunch and unmanned Poster viewing	12:30-13:30	
Emerging Technologies in Gene and Cell Therapy Chairs Dr Kelly Martinovich and Dr. Hilary Sheppard	13:30-14:15	Invited Speaker – Prof. Angus Johnston
	14:15-14:30	Abstract talk 15 – Dr. Anne Klein *AI-Guided Design of Gene Therapy Vectors: Integrating Cargo and Capsid Modelling Capsid Modelling*
	14:30-14:45	Abstract talk 16 – Prof. Sharon Ricardo *Matrix rigidity amplifies lysosomal dysfunction and fibrotic signalling in Fabry disease iPSC-derived podocytes*
Prizes + Awards	14:45-15:15	A/Prof. Samantha Ginn and Prof. Paul Gregorevic
Greg Johnson Memorial Oration Chair: Dr. Jim Vadolas and A/Prof. Samantha Ginn	15:15-16:00	Invited Speaker – Prof. Andrew Elefanty *Generation of long-term engrafting haematopoietic cells from human induced pluripotent stem cells*
End of Meeting	16:00	

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Melbourne Connects highly experienced team will work tirelessly to bring the AGCTS meeting to life, with dynamic meeting rooms. Located within the Melbourne Connect precinct, Atlantic Group® offers a comprehensive catering solution for events of all sizes. Their professional team ensures every detail is taken care of, from thoughtfully designed menus to innovative onsite experiences.

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B. Sponsors

The AGCTS Executive Committee would like to acknowledge and thank our sponsors for their generous support of our biennial meeting:

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C. Invited Speaker Biographies

Professor Damjan Osredkar

University Children's Hospital in Ljubljana, Slovenia



Prof. Damjan Osredkar, MD, PhD is the head of the Pediatric neurology department of the University Children's Hospital in Ljubljana, Slovenia. Prof. Osredkar received his degree at the University of Ljubljana in 1999 and his PhD in 2006. He has been awarded with Fulbright Scholarship in 2006 and has worked as a postdoctoral fellow at the University of California San Francisco, USA (2007-2009) and University of Oslo, Norway (2012-2014). His areas of interest include novel treatment options, gene therapy, translational science, neuromuscular diseases, neuroprotective treatment. He is the PI of the clinical trial of gene augmentation therapy for the CTNNB1 neurodevelopmental syndrome (NCT07270549).

Professor Krystof Bankiewicz

Ohio State University, Ohio, United States.



Dr. Krystof Bankiewicz earned an M.D. from Jagiellonian University in Kraków, Poland, and a Ph.D., D.Sc. from the Institute of Neurology and Psychiatry in Warsaw, Poland, as well as the title of Professor by the President of the Republic of Poland. He is a member of the Polish Academy of Science, Polish Academy of Arts and Science and US National Academy of Inventors. Dr. Bankiewicz was a recipient of a Fogarty Fellowship and trained under Dr. Irvin Kopin and Dr. Edward Oldfield at the Surgical Neurology at National Institutes of Neurological Disorders and Stroke at National Institutes of Health (NIH), Bethesda, Maryland, where he remained as a Staff Scientist.

Currently, Dr. Bankiewicz is Tenured Professor of Neurological Surgery and the Gilbert and Kathryn Mitchell Endowed Chair Director of the Brain Health and Performance Center and Chief Scientific Officer of the Gene Therapy Institute at The Ohio State University. Dr. Bankiewicz is Director, Interventional Neuro Center at Brodno Hospital in Warsaw, Poland. He is also Scientific Chairman of Parkinson's and Multiple System Atrophy Gene Therapy program at AskBio/Bayer Therapeutics. Dr. Bankiewicz has retired after 30 years from position of Endowed Chair of the Kinetics Foundation in Translational Research and Tenured Professor of Neurological Surgery at the University of California, San Francisco (UCSF).

Among his many professional experiences, it is notable that Dr. Bankiewicz held the positions of Chief of Molecular Therapeutics Section and Chief of CNS Implantation Unit, Surgical Neurology Branch, NINDS at NIH and has held a variety of esteemed positions related to neurology and neurosurgery at UCSF. Dr. Bankiewicz has additionally been a recipient of research grants from multiple organizations, including NIH, DOD and CIRM.

Dr. Bankiewicz is a recognized expert in neuro-restorative medicine, including delivery of therapeutics to the brain as well as gene therapy with successful translation of multiple drugs and gene therapies to the clinic. He has industry and academic experience, is an inventor on numerous patents and has published more than 250 peer-reviewed research articles.

Throughout his career, he has maintained a strong focus on the development of translational approaches to drug, gene and cell replacement therapies, and has displayed the ability to synthesize distinct technologies into powerful new approaches to the treatment of serious diseases, including brain cancer and neurodegenerative disorders of the brain. Dr. Bankiewicz co-founded several biotechnology and gene therapy companies. He pioneered ongoing gene therapy clinical trials in Parkinson's Disease, Multiple System Atrophy, Alzheimer's Disease and in pediatric neurotransmitter deficiency. He also invented several devices currently used clinically to administer gene therapy to the brain.

Sheila Mikhail JD, MBA
M34 Inc. and Columbus Children's Foundation



Sheila Mikhail has almost 25 years of biopharmaceutical leadership experience. She is a serial entrepreneur, an advocate for equitable access to healthcare, and a philanthropist.

Sheila co-founded and ran several companies, including AskBio (sold to Bayer in 2020 in a $4B transaction) and Bamboo Therapeutics, Inc. (sold to Pfizer in 2016 in a deal valued at $827M). She was also a founder and part of the management team of Chatham Therapeutics, LLC (sold to Baxter in 2014) and Viralgen VectorCore S.A., a gene therapy CDMO business (sold to Bayer in 2021). In further pursuit of expanded global access to healthcare, Ms. Mikhail recently co-founded Jurata Thin Film, Inc., to eliminate the need for cold chain storage requirements.

Committed to ensuring that gene therapy treatments are developed for patients suffering from ultra-rare diseases, in 2017, Ms. Mikhail co-founded Columbus Children's Foundation (CCF). To date, CCF has supported the treatment of over 50 children suffering from amino acid decarboxylase deficiency (AADC), a devastating neurological disease with no viable treatment options.

Following her breast cancer diagnosis in 2022, Ms. Mikhail formed BC-Ruckus to advocate for expanded access to supplemental screening for women with dense breasts so everyone's breast cancer can be found early.

Ms. Mikhail was recognized as the Ernst & Young 2021 Overall National US Entrepreneur of the Year.

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Professor R. Jude Samulski

M34 Inc. and The University of North Carolina at Chapel Hill



R. Jude Samulski is the Alumni Distinguished Professor of Pharmacology at the University of North Carolina School of Medicine, the Founder and former Director of the UNC Center for Molecular Medicine (formerly the UNC Gene Therapy Center), and co-founder and Chief Scientific Officer of M34 Inc. He is also co-founder and former Chief Scientific Officer of AskBio (now part of Bayer), and co-founder of the Columbus Children's Foundation, whose mission is to accelerate AAV-based therapies for children who have ultra-rare genetic disorders.

Widely regarded as a founding pioneer of modern gene therapy, Dr. Samulski was the first scientist to clone adeno-associated virus (AAV) for therapeutic use. He has dedicated his career to advancing the basic science and translational capabilities of AAV-based vectors, enabling the field of AAV gene therapy to take shape. Accordingly, all thirteen AAV-based gene therapies currently approved utilize Dr. Samulski's technology, with hundreds more in clinical development. Further, with more than 350 published scientific articles, Dr. Samulski is the most highly cited and co-cited scholar in gene therapy and AAV vectors (Jiang et al., *Front Cell Infect Microbiol* 2023). Additionally, he holds more than 300 patents.

Dr. Samulski is the only scientist to receive a Founders Award from leading gene and cell therapy societies across Asia, Europe, and North America (ESGCT in 2022; ASGCT and CSGCT in 2026). In 2018, he was recognized by Pope Francis with an invitation to the Vatican for his work on the treatment of Canavan disease. Dr. Samulski received his B.S. in microbiology in 1976 from Clemson University and his Ph.D. in medical microbiology and immunology in 1982 from the University of Florida.

Dr Adrian Veres

Dyno Theraputics



Adrian Veres is a scientist and entrepreneur building genetic technologies to improve human health. As Chief Scientific Officer and Cofounder of Dyno Therapeutics, he leads Dyno's work to create powerful, specific and safe gene delivery vectors. Adrian and the Dyno team have launched field-leading AAV capsids that unlock new possibilities for CNS, Muscle and Eye gene therapies. In his academic work, Adrian worked with Prof. Douglas Melton and Semma Therapeutics to make human islets from stem cells to treat type 1 diabetes. He holds an MD with Honors from the Harvard-MIT HST program, a PhD in Systems Biology from Harvard University and an AB summa cum laude in Chemistry and Physics from Harvard College.

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Professor P. Joy Ho

University of Sydney and Royal Prince Alfred Hospital,



Prof P. Joy Ho is Professor, Alan Ng Chair in Haematology, University of Sydney, Head of Department & Director of Research at the Institute of Haematology, Royal Prince Alfred Hospital, Sydney, Australia, where she heads the chimeric antigen receptor (CAR)-T Program and Myeloma Unit. She is Founding Director of the Sydney Blood Cancer Research Institute, co-chair of the Myeloma Working Group of the Australasian Leukemia and Lymphoma Group (ALLG). For the American Society of Hematology (ASH), she is the current Chair of the International Members' Committee, since 2024. She is Foundation Member and was Deputy Chair of the Medical and Scientific Advisory group of Myeloma Australia, and on the Steering Committee of the Australasian Myeloma Research Consortium (AMaRC) and the Myeloma and Related Diseases Registry (MRDR). She has been a member of the International Myeloma Working Group (IMWG) since its inception. In 2019, she was elected to the Board of the International Myeloma Society in 2019. She was President of the Haematology Society of Australia and New Zealand (HSANZ) in 2013-15. In 2017-2021, she was appointed to the Pharmaceutical Benefits Advisory Committee by the Australian Government.

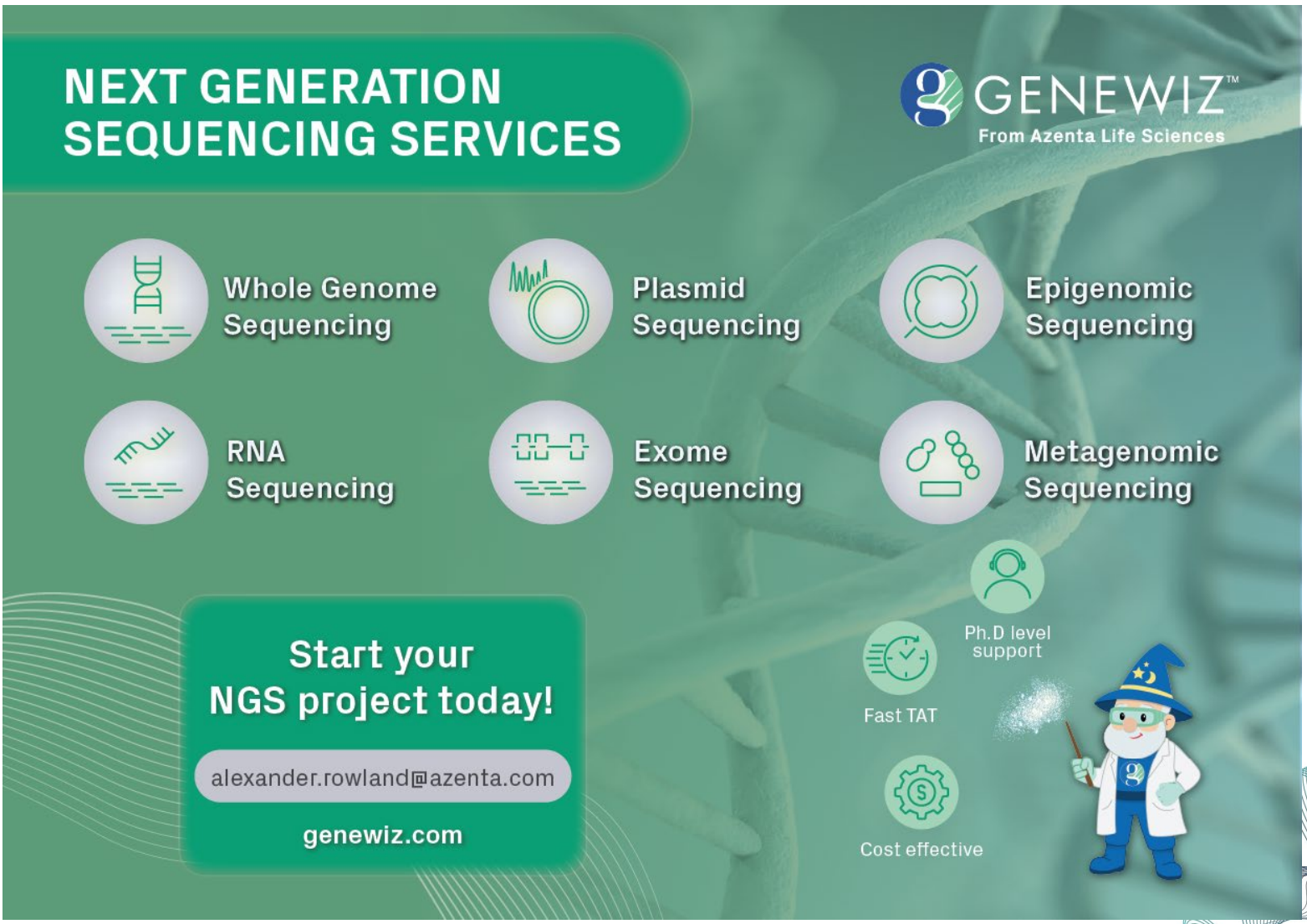
She is principal investigator in the *in vivo* chimeric CAR-T cell therapy trial of KLN-1010 in myeloma, and presented the first results in a late-breaking abstract in ASH 2025, and an update in ASCO 2026. This clinical experience has provided key insights into the feasibility, safety and efficacy of *in vivo* CAR-T in myeloma. Her research achievements include stewardship of national clinical trials, and has brought multiple trials on novel CAR-T, myeloma and lymphoma therapies to Australia. She served on the ALLG Scientific and Executive Committees, and chaired the Laboratory Science Committee. Awards received include the Carl de Gruchy Oratorship (HSANZ) in 2022, Inaugural HSANZ Leadership Award in 2023, Albert Baikie Memorial Medal, Kanematsu Memorial Award, and Oxford Nuffield Medical Fellowship.

Dr Jiang-Hui (Sloan) Wang

Centre for Eye Research Australia and University of Melbourne



Dr Jiang-Hui (Sloan) Wang is principal investigator and head of the Ocular Genetic Therapeutics Research Unit at the Centre for Eye Research Australia and the University of Melbourne. Trained under Professor Guangping Gao at UMass Chan Medical School in the U.S., his work focuses on AAV capsid engineering and translational retinal gene therapy. His program develops next-generation ocular gene delivery platforms and therapeutic strategies for inherited retinal diseases and optic neuropathies, with a particular interest in vector engineering for efficient outer retinal cell targeting via different routes of administration.



A/Prof. Gavin Knott

Monash Biomedicine Discovery Institute



A/Prof. Gavin Knott is a Snow Fellow, Scientific Director of the AI Protein Design Program, and Head of the Nucleic Acid Sensor Laboratory in the Monash Biomedicine Discovery Institute. Gavin obtained his PhD from the University of Western Australia in 2016 where he studied long non-coding RNA-binding proteins involved in human innate immunity and paraspeckle formation. Gavin completed his post-doctoral training at the University of California Berkeley under the supervision of Prof. Jennifer Doudna. During this time, he described the mechanisms of RNA targeting CRISPR tools, systems to control CRISPR activity, and the structural basis for precision CRISPR-Cas9 base editors. In recognition of his contributions, Gavin has received awards and fellowships including the Sir. Keith Murdoch Fellowship from the American Australian Association (2018), the Australian Society of Biochemistry and Molecular Biology Boomerang Award (2019), the Lorne Protein Anders Young Investigator Award (2019), NHMRC Investigator Grant (2020), and Sparrow Award (2026). Currently funded by Snow Medical, his research program aims to discover and design CRISPR or associated nucleic acid sensing systems for application as tools in nucleic acid biotechnology.

Professor Kate Quinlan

University of New South Wales



Kate Quinlan is a Professor at the School of Biotechnology and Biomolecular Sciences, UNSW Sydney. She received her PhD from the University of Sydney in 2006 and, following postdoctoral appointments at The Children's Hospital at Westmead and the University of Cambridge, established her research group focussed on mammalian gene regulation at UNSW Sydney in 2018. Along with a dedicated team of postdocs, PhD students and honours students, Kate is exploring how gene editing and epigenome editing can be used to model disease and seek new therapeutic options for genetic blood diseases.

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Dr. Christina Cortez-Jugo

The University of Melbourne



Dr. Christina Cortez-Jugo is a Senior Research Fellow at The University of Melbourne. She has a PhD in Chemical Engineering from The University of Melbourne, with postdoctoral training at Massachusetts Institute of Technology (Langer Lab) on nanoparticle platforms for gene delivery and at Monash University as an ARC Super Science Fellow. As an engineer, she collaborates widely with clinicians and life scientists on targeted delivery approaches toward neurodegenerative and respiratory diseases, including infectious diseases. She specialises in nanoparticle engineering, including the development of novel nanomaterial platforms for the delivery of genetic cargo and small molecule drugs to specific sites (e.g., tumour, lung, nervous system) with funding from the NHMRC, ARC, Friedreich's Ataxia Research Alliance, and Australian Centre for HIV and Hepatitis Virology Research.



Professor Angus Johnson

Monash Institute of Pharmaceutical Sciences



Prof Angus Johnston is the head of the Nanomaterials for Biology group, who's work focuses on developing better ways to deliver drugs, making them more therapeutically active and limiting side effects. He has a particular interest in developing targeted mRNA therapeutics and the translation of fundamental science into clinically relevant therapeutics. He has extensive knowledge and expertise in nanomaterials assembly, material characterisation, cellular interactions and advanced imaging techniques.

His group is interested in understanding how nanoengineered materials interact with biological systems. This work involves developing molecular sensors to determine the internalisation, trafficking and local environment that nanoparticles are exposed to in living cells. With a better understanding of how these materials interact with cells, we can engineer smarter materials for improved drug and vaccine delivery.

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Professor Andrew Elefanty

2026 Greg Johnson Orator

Murdoch Children's Research Institute



Professor Andrew Elefanty is the Group Leader of the Blood Diseases Laboratory at the Murdoch Children's Research Institute (MCRI). Prof Elefanty's research focuses on the differentiation of human pluripotent stem cells to understand and model blood diseases in vitro and for transplantation. Together with the laboratories of Professor Ed Stanley (Immune Development group) and Dr Elizabeth Ng (Blood Development group) at MCRI, Prof Elefanty has made important contributions to the generation of genetically modified human stem cell lines in which lineage-specific fluorescent reporters allow monitoring of differentiation.

After training as a physician, Professor Elefanty completed a PhD in leukaemogenesis at the Walter and Eliza Hall Institute of Medical Research supervised by Professor Suzanne Cory. He subsequently worked on globin gene regulation with Professor Frank Grosveld at the National Institute for Medical Research in Mill Hill, London before returning to the Hall Institute to pursue interests in developmental haematopoiesis and the differentiation of mouse embryonic stem cells.

He moved to Monash University in 2002 to initiate studies with human embryonic stem cells. In 2013, his laboratory relocated to the Murdoch Children's Research Institute. In collaboration with Dr Elizabeth Ng and Prof Ed Stanley, he has focused on haematopoietic differentiation of human pluripotent stem cells.

Steve Hille

FOXG1 Research Foundation



Steve Hille, father of Mina, is a passionate rare disease advocate and CEO of the FOXG1 Research Foundation. Steve currently serves as Co-Chair of the Leadership Group for the Rare Care Comprehensive Centre and Co-Lead of the Treatments and Trials Flagship, helping to drive strategic initiatives that improve access to clinical trials and emerging therapies for individuals and families affected by rare diseases. He is also a member of the Executive Advisory Group for TrialR, the Rare Care Clinical Centre of Expertise for Rare and Undiagnosed Diseases (RUD), where he contributes consumer and patient-focused perspectives to trial readiness and research programs

A strong advocate for patient involvement in research, Steve serves as an Advisor to the Rare Trials Alliance Platform of Platforms Clinical Trials Infrastructure and has provided consumer advice on numerous grant applications developed by researchers at the Personalised Medicine Centre at Murdoch University. In recognition of his advocacy and leadership, he was invited to speak at the Telethon Gift of Giving event in 2024.

Through his work across multiple national and international initiatives, Steve continues to champion patient-centred research, equitable access to innovative therapies, and stronger partnerships that improve outcomes for people living with rare diseases and their families.

Emma Bonser

Genetic Alliance Australia



Emma Bonser is Chief Executive Officer of Genetic Alliance Australia, the national umbrella organisation for people living with rare, genetic and undiagnosed conditions. She combines 20 years' professional experience across health, strategy and advocacy with more than two decades as a family carer.

Emma advocates for equitable access to diagnosis, treatment and ongoing care, and works to ensure patient and family priorities shape research, policy and services. She holds investigator and advisory roles across national genomics and health system initiatives and is an Adjunct Associate Lecturer in the Discipline of Paediatrics and Child Health at UNSW Sydney. She is particularly passionate about making sure that advances in innovation reach ultra and nano rare communities as equitably as possible.

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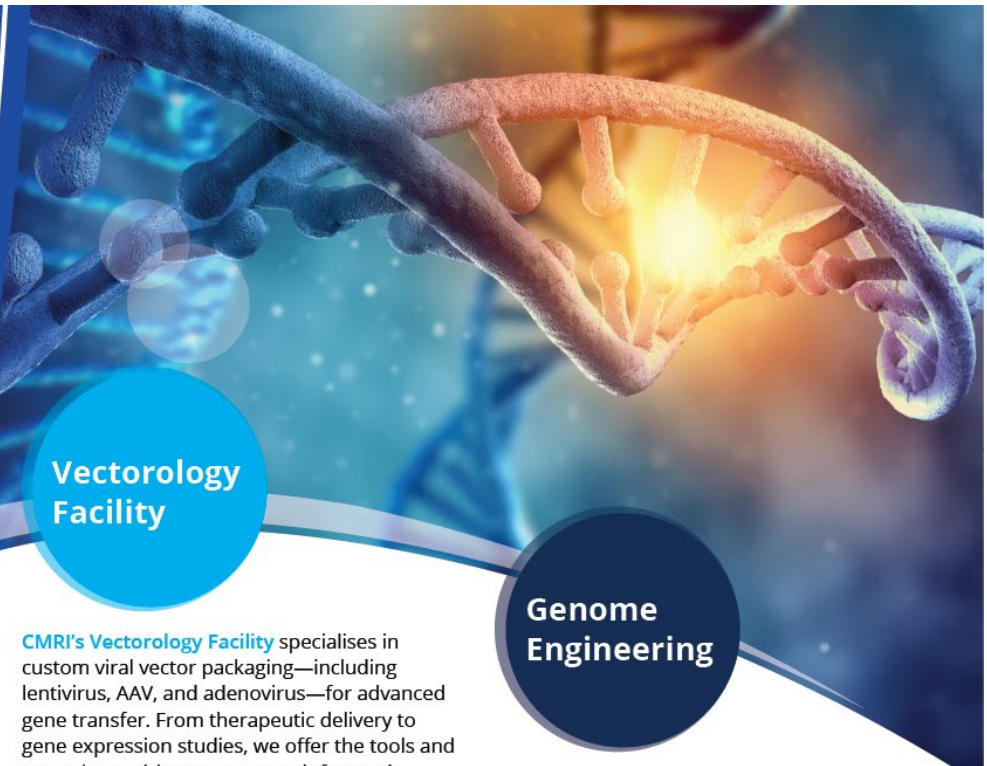
RESEARCH FACILITIES

Children's Medical Research Institute (CMRI) is world-renowned for our work on cancer, gene therapy, telomeres, and stem cells. We operate multiple research facilities to help advance our cutting-edge discovery and translational research but also to benefit our research collaborators and the wider scientific community.

Your research Accelerated

Josh Studdert
Research Facilities Manager
Ph: 02 8865 2965
E: jstuddert@cmri.org.au

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Professor Leszek Lisowski

Children's Medical Research Institute



Professor Leszek [pronounced LESHEK] Lisowski is an internationally recognised expert in gene therapy and vectorology, with over 20 years of experience in the development, manufacturing, and translation of viral vector–based technologies, particularly adeno-associated virus (AAV) therapeutics. He is Professor of Gene Technology and Therapeutics at The University of Sydney and Head of the Translational Vectorology Research Unit at Children's Medical Research Institute (CMRI), where he leads a multidisciplinary program developing next-generation viral vectors for clinical application.

Following his PhD at Cornell University and postdoctoral training at Stanford University, Prof. Lisowski was recruited to the Salk Institute for Biological Studies in La Jolla, California, where he led the Gene Transfer, Targeting and Therapeutics Facility, while also completing an MBA at the UC San Diego Rady School of Management.

Since joining CMRI and The University of Sydney in 2015, his laboratory has gained international recognition for pioneering work in AAV biology, AAV bioengineering, and translational gene therapy. His work has advanced AAV technologies from discovery to clinical translation, including the development of the first bioengineered AAV vector to enter human clinical trials, establishment of Australia's clinical-grade viral vector manufacturing capabilities, and development of the first AAV-based therapy for CTNNB1 Syndrome.

Prof. Lisowski has played a central role in establishing Australia's gene therapy ecosystem, including translational pipelines and cGMP-compliant manufacturing infrastructure. Building on his dual PhD/MBA training, he has co-founded five therapeutic companies and serves as Gene Therapy Advisor to 4BIO Capital. He is also President and co-founder of the Gene2Cure Foundation, a non-profit organisation dedicated to developing and translating gene therapies for rare paediatric genetic disorders.

Professor Robyn Langham AM

Therapeutic Goods Administration



Professor Robyn Langham is the Chief Medical Adviser of the Therapeutic Goods Administration in Australia. Since graduating in Medicine from Monash University, she trained in nephrology in Melbourne and Oxford. A PhD studying the molecular drivers of renal disease led to a career as a clinician researcher, with a focus on translational studies, part of a team developing antifibrotic agents for use in kidney disease. Prof Langham was active in national and international initiatives in combating kidney disease and was the Director of the Nephrology Unit at St Vincent's Hospital in Melbourne. She is an adjunct Professor of Medicine at both Monash University and the University of Melbourne.

Prof Langham is currently the Chair of the Human Research and Ethics Committee of the Royal Children's Hospital and is a Director of the Australian Medical Council. In 2022 she was awarded an Order of Australia for her contributions to renal research and medical education.

Dr Livia Carvalho

The University of Melbourne



Dr Carvalho is a mid-career researcher and Senior Lecturer in the Department of Optometry and Vision Sciences at the University of Melbourne, and an Honorary Research Fellow at the Lions Eye Institute/University of Western Australia, where she leads the multi-institutional Retinal Genomics and Therapy group. She obtained her PhD from University College London in 2009 and completed subsequent postdoctoral fellowships at UCL and Harvard Medical School and was later awarded an Australian Research Council Discovery Early Career Researcher Award (DECRA). Dr Carvalho's research focuses on developing translational platforms for gene and neuroprotection therapies using drug and AAV technologies, and on interrogating the cellular and molecular mechanisms of inherited and untreatable vision loss. Her work has produced 67 peer-reviewed publications with over 4,400 citations and an h-index of 33, reflecting a sustained translational impact and driving preclinical developments to one day make a real difference to patients undergoing vision loss.

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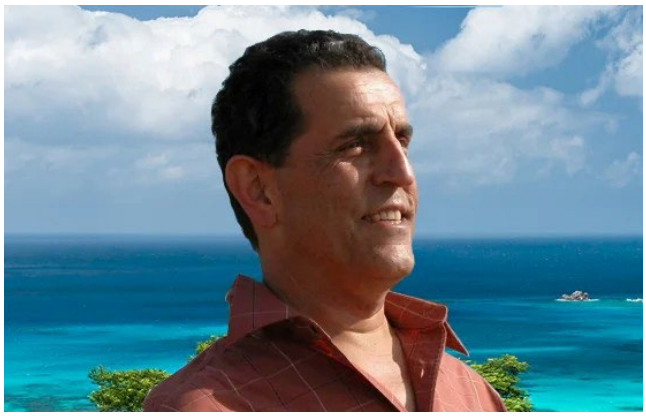
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D. Panos Ioannou New Investigator Award Session

Panos Ioannou
New Investigator Award

This named New Investigator Award was established in 2005 to celebrate the life, commitment and passion of Professor Panos Ioannou (1951-2005). Panos was a founding member of the AGCTS who dedicated his career to developing novel therapies for thalassaemia and other inherited diseases. Up to 8 finalists are selected from submitted abstracts to present in the Panos Ioannou New Investigator Award session. The award is then announced at the biennial AGCTS Conference after each presentation has been assessed by a panel of judges. Prize to be used for travel support to attend a Gene and Cell Therapy Conference in the 2 years after the award.



Name	Affiliation	Title
Aidan Murphy	Murdoch University	Therapeutic Screening of DYRK1A Targeting Antisense Oligonucleotides in Human Neuronal Model for Alzheimer's Disease
Alexander Wykes	University of Sydney	Splicing together Rett syndrome: A multi-isoform, single-AAV vector gene therapy approach
Elizabeth Kalotay	University of New South Wales	AAV-mediated DARS1 gene supplementation for hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL): preclinical proof-of-concept in novel mouse models
Emily Ronning	Centre for Kidney Research, Kids Research	Crystallising Gene Therapy for Primary Hyperoxaluria Type 1
Eva van Dijk	Children's Medical Research Institute	Challenges to durable genome editing in a rapidly renewing tissue
Hsien-Yin Kao	University of Sydney	Rewriting NF: Precision Correction of Nonsense Mutation Using Base Editing and Prime Editing
Natsuki Sasaki	Westmead Institute for Medical Research	Discovery of naturally selected human cardiotropic adeno-associated virus to overcome translational barriers for the use in cardiac gene therapy
Isabella Trew	Murdoch University	Knocking down integrin alpha-4 expression: RNase H-mediated versus splice modulating

Aidan Murphy, PhD Candidate

Murdoch University



Aidan Murphy is a motivated neuroscientist and PhD candidate at Murdoch University's Personalised Medicine Centre, driven by the goal of developing targeted therapies for neurological conditions with limited treatment options. His research sits at the intersection of RNA-based medicine, stem cell biology and neurodegenerative disease, with a particular focus on antisense oligonucleotides for Down syndrome and Alzheimer's disease.

During his PhD, Aidan independently identified and pursued a new therapeutic direction, expanding his research from Down syndrome into Alzheimer's disease. He uses human stem cell-derived neurons to investigate RNA-based therapeutic approaches in disease-relevant cells and has developed expertise across antisense oligonucleotide development, neuronal differentiation and molecular analysis.

Aidan has built collaborations across neuroscience, stem cell biology and RNA therapeutics, broadening the scope and translational direction of his research. He also contributes to the training of students and researchers in stem cell culture and molecular techniques.

As he approaches the completion of his PhD, Aidan aims to continue developing in the translational neuroscience field and contribute to moving RNA-based discoveries closer to clinical application.

Therapeutic Screening of DYRK1A Targeting Antisense Oligonucleotides in Human Neuronal Model for Alzheimer's Disease

Aidan Murphy[1,2], Kelly M. Martinovich[1,2], Steve Wilton[1,2], May Aung-Htut[1, 2]

[1] Personalised Medicine Centre, Murdoch University, Perth Australia

[2] Perron Institute for Neurological and Translational Science, University of Western Australia, Perth, Australia

Introduction: Approximately 446,000 Australians live with dementia, most commonly caused by Alzheimer's disease. Despite decades of research, over 98% of Alzheimer's disease clinical trials have failed, highlighting the need for new therapeutic approaches. DYRK1A is implicated in Alzheimer's disease and is overexpressed in Down syndrome, a population with an elevated lifetime risk of dementia. Although small-molecule DYRK1A inhibitors have been investigated, modulation of *DYRK1A* at the RNA level remains an underexplored approach. Down syndrome-derived neurons may show Alzheimer's disease-associated phenotypes earlier in vitro, enabling comparison with unaffected and Alzheimer's disease-derived cultures. This study aimed to develop a human neuronal platform across these three backgrounds and use it to test *DYRK1A*-targeting antisense oligonucleotides (ASOs).

Methods: Six induced pluripotent stem cell lines, two from each donor background, were differentiated into neural progenitor cells and cortical neurons using an optimised BrainPhys workflow. Neuron identity was confirmed by immunofluorescence for MAP2, TUBB3, NeuN and SYN1. Neural progenitor cells and neurons were treated with *DYRK1A* targeted ASOs using electroporation or lipid-based delivery. *DYRK1A* RNA and protein were assessed using RNA sequencing, RT-PCR and western blot. Additionally, we examined downstream Alzheimer's disease related proteins, phosphorylated-tau, tau, APP and phosphorylated-APP.

Results: Candidate ASOs reduced DYRK1A protein by approximately 90% in neural progenitor cells, with corresponding RNA reduction. Transcriptomic analysis supported the specificity of the candidates. Following treatment before neuronal differentiation, *DYRK1A* suppression remained detectable in differentiated neurons. Direct treatment of four-week-old neuronal cultures reduced *DYRK1A* RNA by 30-40%, with similar protein-level changes. Preliminary day-21 analyses indicated increased total tau and tau phosphorylated at Ser202/Thr205 in Down syndrome-derived neurons relative to unaffected and Alzheimer's disease-derived cultures.

Conclusions: These findings demonstrate that DYRK1A expression can be modified in human neural models and support the potential use of Down syndrome-derived neurons as a model of early-emerging Alzheimer's disease-associated pathology.

Dr Alex Wykes, Postdoctoral Researcher

University of Sydney and Children's Hospital at Westmead



Dr Alex Wykes is a postdoctoral researcher at the University of Sydney and Children's Hospital at Westmead, where he leads efforts to develop novel gene therapy strategies for rare neurodevelopmental and epileptic encephalopathies, including Rett syndrome and CDKL5 deficiency disorder. His research pioneers comparative genomics- and machine learning-based approaches to inform gene therapy design, alongside CRISPR/Cas9 gene editing therapies and multiomics-based biomarker discovery to guide translational development. Central to his current research are novel multi-isoform gene therapy initiatives which address a major unmet challenge in the field, where the multiple isoforms produced from many disease-associated genes have previously been challenging to deliver at physiological levels due to vector packaging constraints. Dr Wykes completed his PhD at the University of Melbourne and the Florey Institute, where he developed novel AAV vector-based molecular tools for targeted neuronal transgene expression in the brain, and applied these to dissecting central arousal and autonomic circuitry. Building on this foundation in precision genetic engineering, he has established independent research interests that integrate evolutionary and comparative genomics with molecular therapeutics, positioning him as an emerging leader in next-generation gene therapy design for neurodevelopmental disorders.

Splicing together Rett syndrome: A multi-isoform, single-AAV vector gene therapy approach vector gene therapy approach

Alexander Wykes[1,2], Riley Dunn[1,2], Adam Bournazos[1,2,3], Nancy Luo[1,2,3], Brian Gloss[4], Florencia Haase[1,2,3], Kelley Gao[1,2], Daniel de los Reyes Helices[1,2,3], Himanshu Joshi[2,3], Sandra Cooper[1,2,3], Wendy Gold[1,2,3]

[1] School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Australia

[2] Kids Research and Kids Neuroscience Centre, The Children's Hospital at Westmead, Australia

[3] Children's Medical Research Institute, Australia

[4] Westmead Research Hub, Westmead Institute for Medical Research, Australia

Background: Rett syndrome is a severe neurodevelopmental disorder caused by loss-of-function variants in the *MECP2* gene. Adeno-associated virus (AAV)-based gene therapies show promise for restoring *MECP2* function. However, despite evidence that both major isoforms (*MECP2*-E1 and *MECP2*-E2) are important to neurodevelopment, current molecular therapies including TSHA-102 (Taysha) and NGN-401 (Neurogene) clinical trials only deliver a single isoform. Here, we aimed to restore physiological *MECP2* isoform expression within the constraints of a single, translationally relevant AAV vector.

Methods: To define physiological *MECP2* isoform expression, E1:E2 ratios were quantified from human frontal cortex RNA-sequencing data spanning prenatal development through adulthood (n=112). An AAV-compatible *MECP2* minigene was engineered by fusing exons 3 and 4 while retaining truncated intronic sequences surrounding the alternatively spliced exon 2. Expression was regulated using EXACT elements adapted from the clinical-stage NGN-401 vector. Isoform ratios were calibrated via intron engineering using our machine-learning platform, GENE*ie*, and screened by PCR following *in vitro* transfection of diverse cell types. Lead constructs were packaged into AAV9 vectors and evaluated following *in vivo* delivery to mouse brain.

Results: Analysis of the human frontal cortex transcriptome revealed a predominance of *MECP2*-E2 during prenatal and early postnatal development, followed by stabilisation at an approximately 1:1 (E1:E2) isoform ratio from childhood onwards, coinciding with the typical age of Rett syndrome symptom onset. Initial minigene constructs drove exclusively *MECP2*-E2 expression. However, GENE*ie*-guided intron engineering successfully achieved stochastic exon 2 skipping, resulting in robust dual-isoform expression at the target physiological 1:1 isoform ratio.

Conclusions: These findings reveal key features of endogenous *MECP2* splicing and demonstrate the feasibility of delivering multiple clinically relevant isoforms from a single AAV vector. In addition to improved gene therapies for Rett syndrome, this strategy provides a broader framework for treating monogenic disorders in which restoration of multiple gene isoforms is therapeutically important.

Dr. Elizabeth Kalotay, Postdoctoral Researcher

University of New South Wales



Elizabeth Kalotay recently completed her PhD in the Nervous System Gene Therapy and Repair Group within the Translational Neuroscience Facility at UNSW Sydney. She is interested in applying fundamental neuroscience knowledge and emerging gene therapy technologies to develop treatments for debilitating neurological diseases for which no disease-modifying therapies currently exist.

Elizabeth joined the group in 2020 to undertake an Honours project investigating a candidate mouse model of the leukodystrophy Hypomyelination with Brainstem and Spinal cord involvement and Leg spasticity (HBSL). She was awarded the Paxinos and Watson Prize for best performance in the Neuroscience Honours program and the University Medal in Neuroscience. During her PhD, and now as a postdoctoral researcher, she has established two novel mouse models of HBSL and used these models to evaluate an AAV-mediated gene supplementation therapy. Her work has resulted in four peer-reviewed publications in neuroscience and gene therapy, including two first-author review articles.

AAV-mediated *DARS1* gene supplementation for hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL): preclinical proof-of-concept in novel mouse models

Elizabeth Kalotay[1], Gary D. Housley[1], Matthias Klugmann[1] & Dominik Fröhlich[1]

[1]Nervous System Gene Therapy and Repair Group, Translational Neuroscience Facility, School of Biomedical Sciences, The University of New South Wales

Background: Leukodystrophies are a large group of genetic diseases that affect the white matter of the central nervous system (CNS). Hypomyelination with Brainstem and Spinal cord involvement and Leg spasticity (HBSL) is a leukodystrophy caused by biallelic loss-of-function variants of the *DARS1* gene, which encodes the cytosolic aspartyl-tRNA synthetase. Mutations in all 36 other aminoacyl-tRNA synthetase (ARS) genes have been linked to disorders that disproportionately affect the CNS. Development of therapies for HBSL and other ARS disorders has been hindered by challenges in modelling loss-of-function of these essential genes *in vivo*. Here, we characterised two novel murine models of HBSL and used them to evaluate adeno-associated virus (AAV)-mediated *DARS1* gene supplementation therapy for HBSL.

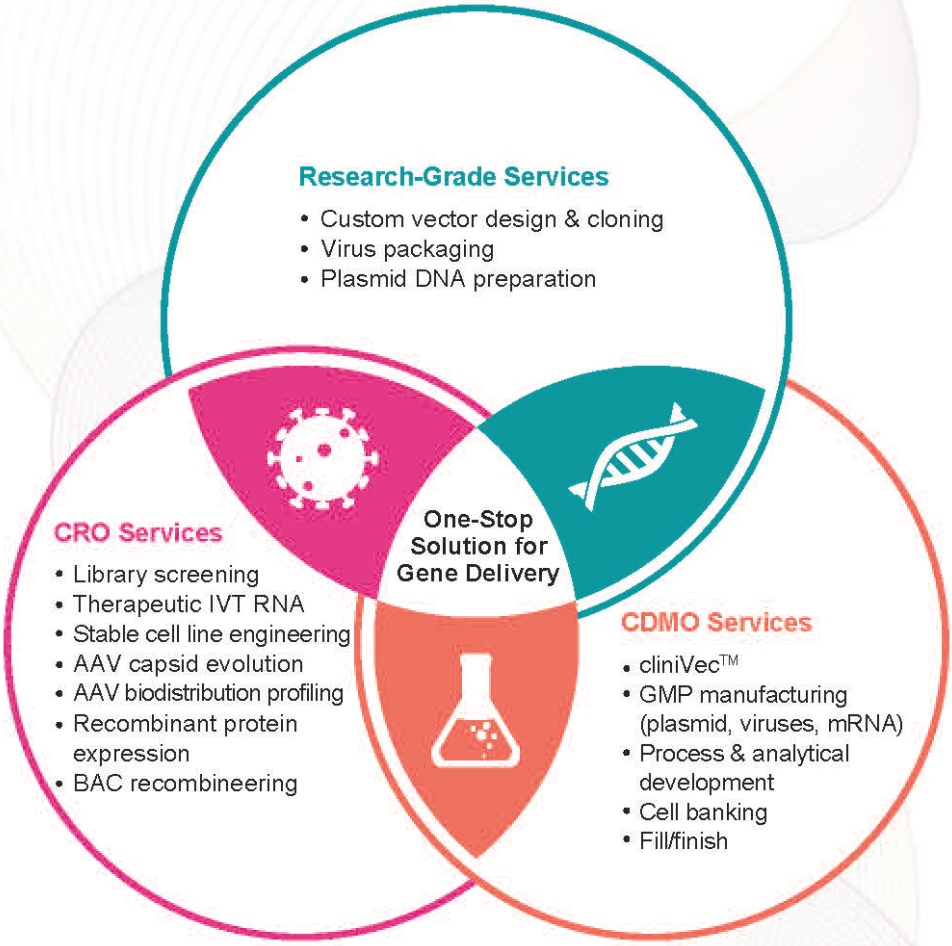
Methods: *Dars1* gene expression was selectively ablated in neurons (*Dars1*^{NeuroKO}) or oligodendrocytes (*Dars1*^{OligoKO}). Models were characterised through behavioural, clinical and physiological phenotyping, alongside histological and molecular analyses. In parallel, PHP.eB AAV vectors were developed to express a codon-optimised, CpG-free human *DARS1* coding sequence (*coCpG*$^{-}$*DARS1*), using promoters with different CNS expression profiles. Therapeutic efficacy was evaluated in both models following systemic AAV administration.

Results: Untreated *Dars1*^{NeuroKO} and *Dars1*^{OligoKO} mice exhibited CNS cell loss, white matter abnormalities and neuroinflammation, accompanied by severe motor dysfunction and markedly reduced lifespan. Treatment of *Dars1*^{NeuroKO} mice rescued motor function and normalised CNS histopathology. Strikingly, neonatal treatment extended median *Dars1*^{NeuroKO} survival from 23 to 251 days. AAV treatment produced modest, non-significant improvements across several outcomes in *Dars1*^{OligoKO} mice.
Conclusions: Long-term rescue of *Dars1*^{NeuroKO} mice by AAV-mediated *coCpG*$^{-}$*DARS1* delivery provides strong preclinical proof-of-concept for this therapeutic strategy for HBSL. Further optimisation of treatment timing and cellular targeting may improve therapeutic efficacy in the *Dars1*^{OligoKO} model. The models and experimental workflow established here may also facilitate therapeutic developments for other ARS disorders and leukodystrophies, where similar challenges must be overcome.

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Thomas Leung, PhD
Associate Director,
Australia and Southeast Asia
m: +61 (0) 481 789 108
e: thomasleung@vectorbuilder.com

Chee Ho H'ng, PhD
Territory Manager,
Australia and Asia Pacific
m: +61 (0) 435 158 225
e: cheehohng@vectorbuilder.com

Emily Ronning, PhD Candidate

Centre for Kidney Research, Kids Research



Emily Ronning is a final year PhD student at the Centre for Kidney Research (CKR), Kids Research. Her research focuses on developing gene therapy for rare genetic kidney disease. She completed an undergraduate degree in Medical Science with honours and is concurrently studying Medicine.

Crystallising Gene Therapy for Primary Hyperoxaluria Type 1

Emily Ronning[1], Jessica Wei[1], Ngan Nguyen[1], Karli Shaw[1], Jennifer Rigterink[1], Samantha L. Ginn[2], Ian E. Alexander[2], Beena Devanapalli[3], Hugh McCarthy[1], Yuan M. Wang[1], Stephen I. Alexander[1]

[1] Centre for Kidney Research, Children's Hospital at Westmead, University of Sydney, Sydney, Australia.

[2] Gene Therapy Research Unit, Children's Medical Research Institute, The University of Sydney and The Sydney Children's Hospitals Network, Westmead, NSW, Australia.

[3] NSW Biochemical Genetics Service, The Children's Hospital at Westmead, Sydney, NSW, Australia.

Background: Primary Hyperoxaluria Type 1 (PH1) is a rare genetic kidney disease. Mutations in the liver-expressed gene, *AGXT*, leads to hyperoxaluria, kidney calcium oxalate crystals and stone formation, this can progress to renal failure requiring liver-kidney transplantation. Historically liver transplantation has been the only curative treatment, however newer RNA interference agents can reduce oxalate but require ongoing monthly injections. Gene editing offers a more permanent therapy. We have developed a gene therapy using Homology Independent Targeted Integration (HITI) to insert a replacement *AGXT* gene and evaluate it in a novel patient avatar mouse model of PH1 (*AGXT* 364C>T, Arg122*). HITI uses a dual AAV *in vivo* editing approach that uses CRISPR/Cas9 to integrate a gene into the affected gene.

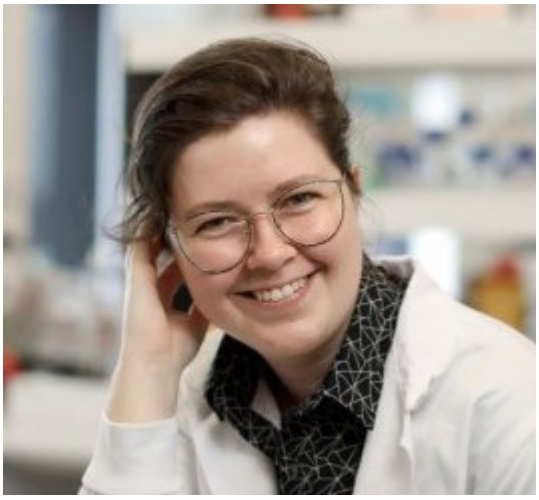
Methods: A dual AAV8 HITI gene therapy was designed to insert a correct *AGXT* gene into the mouse *AGXT* gene. $AGXT^{hom/hom}$ (homozygous) mice received the gene therapy via tail vein injection at 4-6 weeks old ($5x10^{11}$vg per vector/mouse) and were evaluated ~7 months post-injection against age-matched untreated wildtype and homozygous controls. Disease phenotype was assessed using gas chromatography mass-spectrometry for urinary oxalate measurements, micro-CT imaging for kidney/bladder stones, and polarised light microscopy for tissue crystal deposition. Gene therapy delivery was determined using PCR, Sequencing and TaqMan qPCR to calculate vector copy number (VCN).

Results: The VCN of the donor vector relative to *GAPDH* in treated compared to untreated homozygous mice demonstrated an 8.87 x 10^4-fold-change (n=12, **** p<0.0001). Untreated homozygous mice had higher mean urinary oxalate than wildtype mice (203.8% versus 82.42%, n=12, ** p=0.0018), treatment with AAV8 HITI in homozygous mice reduced mean urinary oxalate but not significantly (158.2% versus 203.8%).

Conclusions: HITI-based gene editing therapy was tested in a novel patient avatar mouse model of PH1 with successful hepatocyte transduction and a non-significant reduction in urinary oxalate.

Eva B. van Dijk, PhD Candidate

Gene Therapy Research Unit at the Children's Medical Research Institute



Eva B. van Dijk graduated with a BAdvSci (Hons I) from the University of Technology, Sydney in 2020. Her honours work focused on mass spectrometry-based characterisation of antibiotic resistance in an aquatic bacterial pathogen. She joined the Gene Therapy Research Unit at the Children's Medical Research Institute in 2021, where she spent two years as a Research Assistant working on rAAV-delivered therapies for inherited metabolic liver disease. She began her PhD in 2023, developing an rAAV-LNP genome editing therapy for congenital adrenal hyperplasia.

Challenges to durable genome editing in a rapidly renewing tissue

EB van Dijk[1], LE Graves[1,2,3], E Zhu[1], L Viswanath[1], S Koyyalamudi[3,4], T Wotton[5], S Rosser[5], J Tablizo[5], SL Ginn[1,2], IE Alexander[1,2]

1. *Gene Therapy Research Unit, Children's Medical Research Institute (CMRI) and Sydney Children's Hospital Network, Westmead, NSW, Australia*
2. *Children's Hospital at Westmead Clinical School, University of Sydney, Westmead, NSW, Australia*
3. *Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Westmead, NSW, Australia*
4. *Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia*
5. *NSW Newborn Screening Program, The Children's Hospital at Westmead, Westmead, NSW, Australia*

Introduction: As a therapeutic target, the adrenal cortex is both clinically important and representative of emerging challenges in the field. In the cortex, non-integrative strategies cannot provide durable therapeutic benefit due to continuous tissue regeneration. *In vivo* genome editing in adrenocortical stem cells (AdSCs) is required to durably correct monogenic adrenocortical disorders including congenital adrenal hyperplasia (CAH). Previous work showed durable benefit following dual-rAAV.Rh10 delivery of a templated CRISPR/Cas9 genome editing strategy to a murine CAH model. However, LNP-mRNA delivery of Cas9 is preferable for clinical translation. No prior work has identified AdSC-targeting vectors or investigated rAAV-LNP genome editing performance in the adrenal cortex, so we aimed to address these gaps.

Methods: Vectors were screened individually in Ai9 mice, where successful Cre delivery to a cell causes permanent, heritable TdTomato expression. Using AdSC-targeting vectors identified, we delivered an rAAV-LNP editing approach to a CAH mouse model. Editing efficiency and phenotypic correction were evaluated. Finally, the role of vector administration timing in adrenocortical editing efficiency was assessed in Ai9 mice.

Results: Screening experiments identified an LNP formulation and multiple rAAV capsids which targeted cells that repopulated the cortex for as long as 52 weeks (4× the complete renewal time). Unexpectedly, when rAAV and LNP were used together to deliver therapeutic editing reagents, adrenocortical editing activity and therapeutic efficacy were negligible, whereas hepatic editing was efficient. Investigating the contribution of inter-vector spacing, we observed improved adrenocortical editing when spacing was shortened from 14 to 7 days.

Conclusions: This work is the first to identify AdSC-targeting vectors and to assess adrenocortical rAAV-LNP genome editing. It directly demonstrates that optimal rAAV-LNP dosage spacing is tissue-specific, highlighting an underexplored challenge in therapeutic development for regenerative tissues. This is critical for future development of genome editing therapies for the adrenal cortex and raises important considerations for other rapidly renewing tissues.

Hsien-Yin Kao, PhD Candidate

University of Sydney



Hsien-Yin Kao is a final-year PhD candidate in the School of Chemical and Biomolecular Engineering at the University of Sydney, based in the Bioengineering & Molecular Medicine Laboratory at the Children's Hospital at Westmead and the Westmead Institute for Medical Research, under the supervision of Associate Professor Aaron Schindeler. Her research focuses on neurofibromatosis type 1 and CRISPR-based therapeutic development, including canonical Cas9 editing as well as base and prime editing. She also works on extensive animal studies aimed at improving AAV delivery efficiency. At AGCTS she will present work comparing base and prime editing for correction of a recurrent NF1 nonsense variant.

Rewriting NF: Precision Correction of Nonsense Mutation Using Base Editing and Prime Editing

Hsien-Yin Kao[1, 2], Alexandra K. O'Donohue[1, 2], Aaron Schindeler[1, 2]

[1] Bioengineering & Molecular Medicine Laboratory, The Children's Hospital at Westmead and the Westmead Institute for Medical Research, Sydney, NSW, Australia

[2] School of Chemical and Biomolecular Engineering, Faculty of Engineering, The University of Sydney, Sydney, NSW, Australia

Background: Neurofibromatosis type 1 (NF1) is a common genetic disorder characterised by tumour formation and multisystem complications. Current treatments for NF1-associated tumours are limited in efficacy and do not address the underlying genetic defect. CRISPR-based gene editing raises the possibility of repairing pathogenic variants to prevent tumour initiation. Base editors (BEs) install precise nucleotide transitions without double strand breaks, while prime editors (PEs) can generate a wider range of small edits are often constrained by efficiency and design requirements. This study compares both platforms for correction of the NF1 c.3916C>T (p.Arg1306*) nonsense variant.

Methods: HEK293T and human Schwann cells (ipn02.8) were engineered to carry NF1 c.3916C>T by Cas9 mediated homology directed repair (HDR), with constructs delivered by plasmid transfection or electroporation. Multiple adenine base editors and pegRNA designs for PEmax mediated correction were evaluated. Editing efficiency, bystander editing and the top 10 predicted off target sites were quantified by next generation sequencing (NGS).

Results: HDR efficiency was sufficient to isolate a homozygous mutant HEK293T clone and a heterozygous ipn02.8 clone (allele 1: c.3916C>T; allele 2: a 3-bp deletion). In HEK293T cells, ABE8e achieved the highest correction efficiency (66%) but generated bystander edits, whereas ABE8eWQ achieved 57% correction with minimal bystander activity. PEmax achieved a mean correction efficiency of 43% across pegRNA combinations with no detectable bystander edits. No editing above the limit of detection was observed at the 10 predicted off-target sites analysed.

Conclusion: ABE8eWQ and PEmax both enable correction of NF1 c.3916C>T, with ABE8eWQ providing higher editing efficiency and PEmax a cleaner bystander-editing profile. Correction is currently being validated in the heterozygous Schwann cell model, which more closely reflects the patient genotype. This model also provides a platform for evaluating Schwann cell targeted AAV and lipid nanoparticle delivery strategies, bridging editor optimisation and translation to NF1-relevant tissues.

Natsuki Sasaki, Postdoctoral Fellow

Westmead Institute for Medical Research



Natsuki Sasaki recently completed her PhD at the Centre for Heart Research, Westmead Institute for Medical Research, The University of Sydney. She is passionate about translational research and has over 14 years of wet-laboratory experience in biomedical research. As a member of a multidisciplinary research team, she spent seven years contributing to the development of a clinical trial evaluating rAAV-mediated gene therapy for ornithine transcarbamylase (OTC) deficiency in paediatric patients. In this role, she was involved in assessing the efficacy and safety of clinical-grade rAAV vectors. Natsuki is currently a Postdoctoral Researcher investigating novel recombinant adeno-associated virus (rAAV) vectors for cardiac gene therapy, building on her doctoral research into human cardiac AAV tropism.

Discovery of naturally selected human cardiotropic adeno-associated virus to overcome translational barriers for the use in cardiac gene therapy

Natsuki Sasaki[1], Cindy Y Kok[12], Ahmad Hammoud[1], Ivy KN Chiang[1], Sara Ballouz[3], Max J Cumberland[1], Jett C Ho[11], Cassandra Malecki[8], Sean Lal[8, 9, 10], Leszek Lisowski[6, 7], Ian E Alexander[4, 5] and Eddy Kizana[1, 2, 11]

[1] *Centre for Heart Research, The Westmead Institute for Medical Research, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia*
[2] *Department of Cardiology, Westmead Hospital, Sydney, NSW, Australia*
[3] *School of Computer Science and Engineering, University of New South Wales, Kensington, NSW, Australia*
[4] *Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia*
[5] *Department of Discipline of Child and Adolescent Health, University of Sydney, Westmead, NSW, Australia*
[6] *Translational Vectorology Research Unit, Children's Medical Research Institute, The University of Sydney, Westmead, NSW, Australia*
[7] *Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine, Warsaw, Poland*
[8] *Cardiovascular Precision Laboratory, The University of Sydney, Sydney, NSW, Australia*
[9] *The Baird Institute for Applied Heart and Lung Surgical Research, Sydney, NSW, Australia*
[10] *Department of Cardiology, Royal Prince Alfred Hospital, Sydney, NSW, Australia*
[11] *Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia*
[12] *The University of Sydney, Sydney, NSW, Australia; Mater Hospital Brisbane, South Brisbane, QLD, Australia*

Introduction: Cardiac gene therapy using recombinant adeno-associated virus (rAAV) vectors faces significant barriers to clinical translation, including achieving high transduction efficiency in the human heart and precisely targeting specific cells within heterogeneous cardiac tissues. Improved tissue and cell-type specificity could enhance therapeutic efficacy, enable lower vector doses, and mitigate off-target effects. We hypothesize that naturally occurring AAV capsids with intrinsic tropism for human cardiac tissue may provide superior vector candidates. This study aimed to identify latent AAV isolates persisting in human cardiac tissue and characterize their cellular tropism in human cardiac models.

Methods: AAV VP1 sequences were identified and validated from cardiac tissues obtained from 58 healthy donors from Sydney Heart Bank. Latent AAV genome levels were quantified using digital droplet PCR (ddPCR). Vector performance was evaluated using barcoded rAAV pools coupled with single-nuclei RNA sequencing (10x Chromium) in human induced pluripotent stem cells (hiPSC)-derived cardiac cells and patient-derived myocardial slices.

Results: Nineteen novel AAV isolates were identified in human ventricular tissue and were phylogenetically related to AAV2(47.4%), AAV9(5.2%), and AAV13(47.4%). No significant differences in AAV prevalence were observed between ventricles or sexes. ddPCR quantification revealed that 30% of samples contained latent AAV genome levels exceeding vector genome copy numbers previously associated with therapeutic efficacy in preclinical SERCA2a cardiac gene therapy studies, supporting the biological relevance of these naturally occurring cardiac AAV isolates. Single-nucleus transcriptomic analysis successfully detected viral barcodes in hiPSC-derived cardiac cells, demonstrating the feasibility of high-resolution assessment of vector cellular tropism. Finally, analyses to evaluate functional transduction efficiency and cell-type-specific tropism in native human cardiac tissue is underway using patient-derived myocardial slices from an individual with desminopathy, a rare genetic cardiomyopathy that may benefit from gene therapy.

Conclusion: Natural cardiac AAV isolates represent promising candidates for the development of next-generation vectors for cardiac gene therapy.

Isabella Trew, PhD Candidate

Murdoch University



Isabella is a final-year Doctor of Philosophy candidate developing patient-derived disease models and precision therapeutics for the rare paediatric neurodevelopmental disorder Malan syndrome. In partnership with the Malan Syndrome Foundation, her research has generated clinically relevant models for therapeutic discovery in Malan syndrome, with a vision of translational centered research that combines mechanistic discovery with therapeutic development to improve outcomes for children living with rare genetic conditions.

Knocking down integrin alpha-4 expression: RNase H-mediated versus splice modulating ASOs

Isabella Trew[1,2], Anuradha Sooda[1,2], Karina Yui Eto[1,2], Mathias Danielsen[3], Kavitha Sudheendran[3], Balázs Schäfer[3], Marvin Caruthers[3], Steve D Wilton[1,2], May T Aung-Htut[1,2]

[1] Personalised Medicine Centre, Health Futures Institute, Murdoch University, Perth, WA 6150, Australia

[2] Perron Institute for Neurological and Translational Science, University of Western Australia, Perth, WA 6009, Australia

[3] Department of Biochemistry, University of Colorado Boulder, Boulder, CO 80309, USA

Background / Introduction: Integrin alpha-4 (ITGA4) is a subunit of T-lymphocyte integrin receptors implicated in hyper-inflammatory responses associated with conditions including Duchenne muscular dystrophy, multiple sclerosis and Crohn's disease. We previously developed a splice-modulating antisense oligonucleotide (ASO) that induces exon 4 skipping, reducing ITGA4 protein expression and delaying disease progression in a mouse model of multiple sclerosis. Here, we investigated alternative chemical modifications of our lead splice-modulating ASO and compared their efficacy and transcriptomic effects with the RNase H-activating gapmer ATL1102 (Avicursen).

Methods: Jurkat T-lymphoma cells were nucleofected with ATL1102 or our lead splice-modulating sequence incorporating PMO, 2′-MOE or a novel chemical modification (1–25 μM). Cells were harvested between 48 hours and 12 days post-treatment to assess *ITGA4* exon 4 skipping by RT-PCR and protein expression by western blotting and flow cytometry. Bulk RNA sequencing was performed to investigate transcriptomic effects.

Results: At 48 hours, the novel chemistry and PMO ASOs demonstrated comparable activity to ATL1102. However, at later time points and lower doses, splice-modulating ASOs demonstrated superior and more sustained ITGA4 knockdown. Cell-surface ITGA4 was reduced by 60% and 58% following treatment with the novel chemistry and PMO, respectively, compared with 36% for ATL1102 and 19% for the splice-modulating 2′-MOE. Additionally, transcriptomic analysis detected substantially fewer differentially expressed genes in cells treated with the novel chemistry and PMO compared with ATL1102 treatment.

Conclusions: Our splice-modulating ASOs demonstrate potent and sustained ITGA4 suppression, with reduced transcriptomic perturbation compared to ATL1102, supporting further development of splice modulation as a targeted approach for ITGA4 suppression.

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Oral 1

CRISPR editing as a strategy for gene therapy for telomere-related bone marrow failure

Ashley Yang[1], Vivian Lieu[1], Karen L. MacKenzie[1], Leszek Lisowski[1], Tracy M. Bryan[1]

[1] Children's Medical Research Institute, Westmead, NSW 2145, Australia

Background: Telomere biology disorders (TBDs) arise from inherited pathogenic variants in genes encoding telomerase components and telomere maintenance proteins, leading to accelerated telomere attrition and depletion of haematopoietic stem and progenitor cells (HSPCs). Bone marrow failure is a major cause of morbidity and mortality in TBD patients, with allogeneic stem cell transplantation remaining the only curative treatment despite substantial complications. Precise gene correction of autologous HSPCs therefore represents an attractive therapeutic strategy for TBDs, but development is challenged by lack of availability of physiologically relevant human stem cell and *in vivo* models for evaluating functional rescue.

Methods: We developed a CRISPR/Cas9-mediated homology-directed repair strategy for editing *TERC*, the gene encoding the RNA subunit of telomerase, in primary human CD34+ HSPCs. Patient-specific variants were introduced to model disease phenotypes, with telomerase activity, telomere maintenance and haematopoietic function assessed *in vitro*. Edited HSPCs were transplanted into NBSGW mice to assess *in vivo* engraftment.

Results: Mean editing efficiency reached ~90% without enrichment and near-complete after enrichment. Our lead sgRNA supports efficient editing of variants, even 250 bp from the Cas9 cut site. Editing was detected in primitive stem cells, with no off-target editing at the top predicted sites. *TERC* transcription and RNA processing were preserved. *TERC*-mutant HSPCs exhibited impaired growth, reduction in colony proliferation, decreased telomerase activity and excessive telomere attrition. Control CRISPR-edited HSPCs successfully engrafted in NBSGW mice, whereas *TERC*-mutant HSPCs showed reduced human bone marrow chimerism compared with the controls, establishing an *in vivo* readout of impaired haematopoietic fitness for future gene correction and rescue studies.

Conclusion: Our study establishes a high-efficiency CRISPR platform for *TERC* editing in human HSPCs and enables functional evaluation *in vitro* and *in vivo*. Together, the HSPC and humanised mouse models provide a foundation for developing autologous gene-correction therapies for telomere-related bone marrow failure.

Oral 2

In vivo editing of the human inactive X chromosome: therapeutic opportunities and epigenomic consequences of AAV-mediated genome engineering

Mawj Mandwie [1], Sharon C Cunningham [1], Erhua Zhu [1], Katherine A Giles [7,8], Samantha L Ginn [1], Eva B. van Dijk [1], Suzanne Scott [10], Anne Klein [3], Maya Sugden [1], Laurence Wilson [3,4], Susan Siew [2,6], Grant J Logan [1], James Kerr [1], Nader Aryamanesh [9], Phillippa C Taberlay [8], Anthony J Cesare [7], Ryan Lister [5], and Ian E Alexander [1 2*]

[1] Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia
[2] Discipline of Child and Adolescent Health, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia
[3] Australian e-Health Research Centre, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
[4] Macquarie University, Department of Biomedical Sciences, Macquarie Park, NSW, Australia
[5] Australian Research Council Centres of Excellence in Plant Energy Biology and Plants for Space, School of Molecular Sciences, The University of Western Australia, Crawley, WA, Australia, Harry Perkins Institute of Medical Research, Nedlands, WA, Australia
[6] Department of Gastroenterology, James Fairfax Institute of Paediatric Nutrition, Sydney Children's Hospitals Network, Westmead, Australia.
[7] Genome Integrity Unit, Children's Medical Research Institute, University of Sydney, Westmead, NSW, Australia.
[8] Menzies Institute for Medical Research, University of Tasmania, Hobart, TAS, Australia
[9] Bioinformatic Group, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia
[10] Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia

Background: Many X-linked disorders affecting females arise from preferential expression of a pathogenic allele due to skewed X-chromosome inactivation (XCI). Therapeutic reactivation of healthy alleles on the inactive X chromosome (Xi) could provide a treatment strategy for X-linked diseases by restoring expression of endogenous functional genes without altering the underlying DNA sequence, including ornithine transcarbamylase (OTC) deficiency, where limited restoration of enzyme activity may be clinically meaningful. However, the feasibility and consequences of manipulating the human Xi *in vivo* remain poorly understood.

Methods: A chimeric mouse-human liver model was established using FRG mice repopulated with primary hepatocytes from a female patient with OTC deficiency exhibiting highly skewed XCI. Adeno-associated virus (AAV)-mediated CRISPR-Cas9 genome editing was used to disrupt the *XIST* locus, a key regulator of XCI maintenance. Editing outcomes, allele-specific *OTC* expression, DNA methylation, and chromatin state were assessed using targeted sequencing and CUT&RUN profiling of histone modifications.

Results: Despite differential methylation at the *XIST* promoter, both active and inactive X chromosomes were similarly accessible to CRISPR-Cas9 editing. *XIST* deletion resulted in limited reactivation of the silenced wild-type *OTC* allele from the Xi, highlighting the complexity of reversing X-chromosome silencing at individual loci. Epigenomic analyses identified distinct effects of vector delivery and Xi perturbation. AAV transduction caused a global reduction in H3K4me3, while *XIST* deletion produced an X chromosome-restricted reduction in H3K27me3 and broader decreases in H3K27ac in edited hepatocytes.

Conclusions: These findings demonstrate that targeted manipulation of X-chromosome silencing can restore expression of a disease-relevant gene from the Xi, supporting Xi-reactivation strategies for OTC deficiency and other X-linked disorders. More broadly, they demonstrate the power of AAV-mediated gene delivery *in vivo* while highlighting its potential to alter the epigenetic landscape of target cells, underscoring the need to consider such effects in the development and clinical application of genetic medicines.

Oral 3

Allele-specific disruption of a patient *COL1A1* stop-loss (readthrough) variant that introduces a cryptic *Sa*Ca9 PAM site

Aaron Schindeler[1, 2], Christal Au-Yeung[1, 3], Oliver Semler[4], Alexandra K O'Donohue[1, 2], Julian Chu[1 ,3], Lucinda R Lee[1, 3], Samantha L Ginn[3, 5], Deborah Barkauskas[6], Dasol Yoo[7], Thomas Cox[7], Marianne Rohrbach[8], Giulio Marcionelli[8], Cecilia Giunta[8], Lidiia Zhytnik[9], Dimitra Micha[9], Craig F Munns[10, 11]

[1] Bioengineering and Molecular Medicine Laboratory, The Children's Hospital at Westmead and the Westmead Institute for Medical Research, Westmead, NSW, Australia.
[2] School of Chemical and Biomolecular Engineering, Faculty of Engineering, The University of Sydney, Camperdown, NSW, Australia.
[3] School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia.
[4] University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Pediatrics, Cologne, Germany.
[5] Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospital Network, Westmead, NSW, Australia.
[6] Microscopy Unit, Garvan Institute for Medical Research, Darlinghurst, NSW, Australia.
[7] Matrix and Metastasis Laboratory, Garvan Institute for Medical Research, Darlinghurst, NSW, Australia.
[8] Connective Tissue Unit, Division of Metabolism and Children's Research Center, University Children's Hospital Zurich and University of Zurich, Zurich, Switzerland.
[9] Human Genetics, Amsterdam UMC, Amsterdam, the Netherlands.
[10] Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia.
[11] Department of Endocrinology and Diabetes, Queensland Children's Hospital, Brisbane, QLD, Australia.

Background: Osteogenesis imperfecta (OI) is a predominantly monogenic connective tissue disorder in which most cases arise from pathogenic variants in *COL1A1* or *COL1A2*. We investigated a severe paediatric OI case carrying a heterozygous *COL1A1* c.4323_4342del (Δ20) stop-loss variant that generates a 103-amino-acid C-terminal readthrough extension. The deletion also creates a novel allele-specific *Staphylococcus aureus* Cas9 (*Sa*Cas9) protospacer adjacent motif (PAM), providing a unique opportunity for selective genome editing of the mutant allele.

Methods: Patient-derived fibroblasts and osteoblasts were used to define the cellular consequences of the mutant α1(I) collagen chain. Complementary CRISPR-engineered human cell and *Col1a1*Δ20 mouse models were generated to investigate disease mechanisms and evaluate an allele-specific *Sa*Cas9 editing strategy directed against the mutation-created PAM.

Results: Analysis of patient cells and engineered models demonstrated that the Δ20 variant disrupts normal collagen biosynthesis, resulting in intracellular retention of misfolded collagen, endoplasmic reticulum stress, and altered osteogenic gene expression. The *Col1a1*Δ20 model reproduced key features of the human disease and supported a dominant-negative mechanism whereby the readthrough α1(I) chain impairs collagen matrix assembly. Exploiting the cryptic PAM introduced by the mutation, *Sa*Cas9 achieved approximately 86% editing of the mutant allele with no detectable modification of the wild-type allele. Editing predominantly generated frameshift mutations predicted to selectively silence expression of the pathogenic readthrough transcript.

Conclusion: The *COL1A1* Δ20 stop-loss variant causes OI through dominant-negative effects on type I collagen production and assembly. Importantly, creation of a mutation-specific *Sa*Cas9 PAM enables highly selective disruption of the disease-causing allele, establishing a proof-of-concept precision genome-editing approach for dominant *COL1A1*-associated OI.

Oral 4

Pre-clinical validation of AAV gene therapy for KCNV2 retinopathy improves visual function

Rabab Rashwan[1,2], Paula I Fuller-Carter[1], Xin Ru Lim[3], Alicia A Brunet[1,3], Annie L Miller[1,3], Yashvi Bhatt[1,3], Rebekah James[1,3], Denise Anderson[4], Valentina Voigt[1], Joao A Paulo[5], Mehdi Mirzaei[6,7], Melissa M Mangala[8], Emilie O Wong[8], Robyn V Jamieson[8,9,10], Anai Gonzalez-Cordero[8], David M Hunt[1,3], Livia S Carvalho[1,3,11],

[1] Lions Eye Institute, Perth, Australia.

[2] Minia University, Minia, Egypt.

[3] Centre for Ophthalmology and Visual Science, The University of Western Australia, Perth, Australia.

[4] INSiGENe Pty Ltd, Perth, Australia.

[5] Harvard Medical School, Boston, USA.

[6] Macquarie University, Sydney, Australia.

[7] ProHeme Diagnostics Pty Ltd, Sydney, Australia

[8] Children's Medical Research Institute, The University of Sydney, Westmead, Australia.

[9] Save Sight Institute, University of Sydney, Sydney, Australia.

[10] Sydney Children's Hospitals Network, Sydney, Australia.

[11] Department of Optometry and Vision Sciences, The University of Melbourne, Melbourne, Australia.

Background / Introduction: Voltage-gated (Kv) potassium channels are critical for neuronal physiology, and their dysfunction can lead to serious consequences. For example, mutations in the silent modulatory Kv8.2 subunit are known to cause irreversible inherited blindness (KCNV2 retinopathy). This is a currently incurable condition that causes lifelong visual loss, reduced visual acuity, photoaversion, night blindness and abnormal colour vision, alongside a distinctive supernormal electrophysiological (ERG) retinal response to light.

Methods: Our therapeutic construct has a codon-optimised human KCNV2 gene under the expression of a photoreceptor-specific promoter and was packaged into two AAV serotypes: AAV8 and Anc80. Vectors were delivered subretinally to Kv8.2 knockout mice at early and late stage of disease at three different doses. Functional visual testing via electroretinogram was done at 4, 8 and 12 weeks post-treatment. At 12 weeks post-treatment, animals underwent behavioural optomotor testing and eyes were collected for histological, flow cytometry, single cell RNAseq and proteomics analysis.

Results: Treated mice exhibited improved ERG responses and correct expression of KCNV2 and its encoded Kv8.2 protein in photoreceptors. Recovery of visually guided scotopic and photopic optomotor responses to wildtype levels was achieved at lower vector doses, highlighting dose-dependent efficacy. Furthermore, treatment of human retinal organoids derived from a KCNV2 patient iPSC line resulted in substantial Kv8.2 protein rescue.

Conclusions: In this study, we demonstrate that AAV-mediated gene replacement therapy delivering a codon-optimised human KCNV2 gene subretinally into Kv8.2 knock-out mice significantly restores retinal function. This work provides the preclinical proof-of-concept for the safety and therapeutic potential of gene therapy for KCNV2 retinopathy, laying a strong foundation for future clinical trials.

Oral 5

Light-guided spatial gene knockout for wAMD treatment

Xinpu Yang[1], Rui Sang[2], Yagiz Alp Aksoy[3], Ewa Goldys[2], Wei Deng[1]

[1] School of Electric Mechanic and Biomedical Engineering, University of Technology Sydney, Ultimo, NSW, 2007, Australia

[2] Graduate School of Biomedical Engineering, Faculty of Engineering, UNSW Sydney, NSW, 2052, Australia

[3] Centenary Institute and Faculty of Medicine and Health, The University of Sydney, NSW, Australia

Background / Introduction: Wet age-related macular degeneration (wAMD) is a leading cause of blindness in older adults, with neovascular AMD driven by VEGFA-mediated choroidal neovascularization (CNV). Current anti-VEGF therapies require frequent intravitreal injections due to persistent VEGFA production, causing unintended exposure of healthy retinal tissue, raising safety concerns and imposing substantial treatment burdens and costs.

Methods: This study developed spatial gene therapy using light-activated liposomes (NanoSyringe) for localised VEGFA knockout. NanoSyringe were loaded with verteporfin and Cas9/sgRNA targeting VEGFA. Near-infrared light (690 nm) activates verteporfin to generate ROS, disrupting the lipid bilayer and enabling spatially controlled cargo release. Knockout efficacy was validated in HEK293 and ARPE-19 cells, with editing efficiency assessed by T7E1, RT-PCR and ELISA. In vivo therapeutic response was further evaluated in a rat model of wAMD using FFA and OCT to assess vascular leakage, CNV and retinal structural changes.

Results: Spatially controlled VEGFA gene editing in ARPE-19 cells, with reduced VEGFA expression confirmed at the DNA, mRNA and protein levels. CRISPR editing induced 45% indel formation at the VEGFA locus, accompanied by a 60% reduction in VEGFA mRNA expression. In the rat model of wAMD, localised light activation produced a therapeutic response, with FFA and OCT demonstrating reduced vascular leakage compared with conventional non-light-activated liposomes.

Conclusions: These findings demonstrate that spatial VEGFA gene editing and therapeutic suppression of wAMD in vivo. By restricting gene therapy to the targeted retinal region with guided light, this approach has the potential to reduce unintended exposure of healthy tissue, minimise treatment-related safety concerns and enable a potentially single-treatment therapeutic strategy. NanoSyringe therefore provides a precise gene therapy platform that could address the safety and treatment burden of current wAMD therapies and enable new therapeutic solution for other forms of age-related macular degeneration with limited or no approved treatments.

Oral 6

Australia's First 50 L Recombinant Lentiviral Vector Manufacturing Platform: Development, Scale-Up and Orthogonal Analytical Strategy

Ken Hsu[1], Manuel DeValle Medina [1], Claire Pierret [1], Minh Phan [1], Kunal Kalra [1], Benjamin Kalma [1], Habib Rahaman[1], Ted Chang[1], Sharon Cunningham [1], Aduba Collins [1], Mikhail Tselebrovskiy [1], Vasily Medvedev[1]

[1] Viral Vector Manufacturing Facility (VVMF), Level 4, Kids Research, The Children's Hospital at Westmead, 178 Hawkesbury Road, Westmead, NSW 2145, Australia.

Background / Introduction: Recombinant lentiviral vectors are central to genetically modified cell therapies, yet sovereign GMP-grade manufacturing capability in Australia remains limited. We aimed to establish a scalable, GMP-aligned recombinant lentiviral vector manufacturing platform and apply an orthogonal analytical strategy to assess product quality and process performance at 50 L scale.

Methods: A suspension HEK293 upstream process using third-generation, four-plasmid transient transfection was scaled from small-scale development through 5 L rocking bioreactors to a 50 L stirred-tank bioreactor and performed across sites. A fully single-use downstream process incorporated clarification, tangential flow filtration (TFF), chromatography, and sterile filtration. The manufactured self-inactivating VSV-G-pseudotyped vector product, intended for *ex vivo* modification of human T cells, was characterized using orthogonal physical and functional titre assays, together with assays measuring host-cell, plasmid, and process derived impurities.

Results: The platform demonstrated consistent cell growth, transfection efficiency, and vector productivity across scales, producing purified vector product of quality suitable for clinical *ex-vivo* cell therapy applications. At 50 L scale, an infectious titre of 6.7×10^7 TU/mL was achieved at harvest with vector recovery exceeding 30% following purification and final filtration. Orthogonal titre assays showed progressive vector enrichment and correlated with downstream recovery trends. Host-cell DNA and protein were substantially reduced through clarification, TFF and chromatography. The proportion of residual DNA fragments below 200 bp increased approximately five-fold, plasmid DNA relative to vector genomes decreased by more than two orders of magnitude, and residual benzonase was below the assay's lower limit of quantitation in the final product.

Conclusions: This work establishes a scalable Australian lentiviral vector platform integrating upstream production, single-use purification and orthogonal analytical characterization. The platform supports future clinical manufacturing, process control strategy development, and local production of clinically relevant lentiviral vectors.

Oral 7

Therapeutic Silencing of TMPRSS6 Attenuates Iron-Driven Inflammasome Activation and Delays Disease Progression in MDS mice

Jim Vadolas[1, 11], Shahla Vilcassim[1, 2], Nuttanan Pholngam[1, 3, 4], Rattanawan Thubthed[1, 5, 6], Tiwaporn Nualkaew[1, 7], Saovaros Svasti[4], Pornthip Chaichompoo[5], Kai Kysenius[8], Peter J. Crouch[8], Sibylle Dames[9], Mona Eisermann[9], Alberto Martinez[10], Ute Schaeper[9], and George Grigoriadis[1]

[1] Centre for Cancer Research, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

[2] School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia

[3] Graduate Program in Molecular Medicine, Faculty of Science, Mahidol University, Bangkok, Thailand

[4] Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand

[5] Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand

[6] Faculty of Medicine, Praboromarajchanok Institute, Ministry of Public Health, Nonthaburi, Thailand

[7] Department of Medical Technology, Walailak University, Nakhon Si Thammarat, Thailand

[8] Department of Anatomy and Physiology and Centre for Muscle Research, The University of Melbourne, Melbourne, Victoria, Australia

[9] Silence Therapeutics GmbH, Berlin, Germany

[10] Silence Therapeutics PLC, London, United Kingdom

[11] Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia

Background: Myelodysplastic syndromes (MDS) are hematopoietic malignancies characterised by ineffective haematopoiesis and risk of transformation to acute myeloid leukemia. Iron overload is increasingly recognised as a pathogenic amplifier of oxidative stress and innate immune activation within the bone marrow niche, yet therapeutic strategies to modulate iron-driven inflammation remain limited. Hepcidin, encoded by *Hamp*, is a liver-derived hormone that regulates iron homeostasis by binding and degrading the iron exporter ferroportin, thereby limiting iron absorption and iron release from macrophages. Hepcidin expression is regulated by BMP/SMAD signalling, whereas the transmembrane serine protease matriptase-2 (*Tmprss6*) negatively regulates this pathway. Targeting the TMPRSS6–hepcidin axis therefore provides a rational strategy to mitigate iron-mediated inflammation and disrupt MDS pathogenesis. This study investigated whether hepatocyte-directed *Tmprss6* silencing using a GalNAc-conjugated siRNA (SLN124) suppresses iron-driven inflammation and disease progression in MDS.

Methods: We utilised a hepatocyte-directed GalNAc-conjugated siRNA (SLN124) to silence *Tmprss6 in vivo*. The NHD13 murine model of MDS and wild-type controls received monthly subcutaneous SLN124 (3 mg/kg) or oral deferiprone (1.25 mg/mL). *Tmprss6* and *Hamp* transcripts were quantified by RT-qPCR. Tissue iron was assessed by LA-ICP-MS. Inflammasome activation was evaluated by quantification of ASC specks in $CD45^+$ BM and splenic cells. Haematological indices and survival were monitored longitudinally.

Results: Iron accumulation was greatest during early disease and was associated with increased inflammasome activation. Tissue iron burden positively correlated with ASC^+ $CD45^+$ cells in both BM and spleen. SLN124 and deferiprone both reduced tissue iron and inflammasome activation however, SLN124 produced a greater and more sustained suppression of ASC specks. Long-term SLN124 treatment delayed anaemia progression and prolonged survival, with 30% of treated mice remaining alive beyond 450 days, whereas all control and deferiprone-treated mice died by day 420.

Conclusions: Hepatocyte-targeted siTmprss6 reduced iron-driven inflammasome activation, delayed haematological deterioration and improved survival in MDS mice.

Oral 8

Flipping the RAS switch: The effect of overexpressing angiotensin-converting enzyme 2 (ACE2) in primary and secondary liver cancer mouse models

Muditha Wijesinghe[1], Anshuli Razdan[1], Amr Allam[2], Lisa Mielke[2], Leszek Lisowski[3], Ian Alexander[3], Vijayaragavan Muralidharan[4], Peter Angus[5] and Chandana Herath[1,4]

[1]Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3168, Australia

[2]Olivia Newton-John Cancer Research Institute, Heidelberg, VIC 3084, Australia

[3]Gene Therapy Research Unit, Children's Medical Research Institute, Westmead, NSW 2145, Australia

[4]Department of Surgery, Austin Health, University of Melbourne, VIC 3084, Australia

[5]Department of Gastroenterology and Hepatology, Austin Health, University of Melbourne, VIC 3084, Australia

Background / Introduction: Liver cancer remains a leading cause of mortality due to high recurrence rates and limited response to therapies. ACE2 drives the protective arm of the renin-angiotensin system. Building on our previous finding that hepatic ACE2 suppresses liver fibrosis, we are now evaluating liver-targeted ACE2 gene therapy in primary and secondary liver cancer models.

Methods: Hepatocellular carcinoma (HCC), representing primary liver cancer, was induced in C57BL/6 mice using diethylnitrosamine (DEN; 8 weekly injections from 2 weeks of age). Mice received a single intraperitoneal injection of liver-specific adeno-associated viral vector (ACE2-AAV) or control vector (HSA-AAV) prophylactically at 12 weeks, and samples were collected at 24 weeks. Secondary liver cancer was established via intrasplenic injection of MC-38 colon cancer cells concurrent with vector administration.

Results: In HCC, median tumour diameter was significantly elevated in untreated ($p<0.05$) and HSA-AAV ($p<0.001$) groups compared to healthy controls, but was non-significant in ACE2-AAV-treated mice. Flow cytometry revealed that ACE2-AAV significantly increased the natural killer T cell population ($p<0.05$) and enriched type-1 conventional dendritic cells. Multivariate analysis showed that populations of immune cells in the liver were significantly influenced by ACE2 and sex of the animals. In the secondary liver cancer model, ACE2-AAV significantly reduced tumour burden, achieving a 33% reduction in relative liver weight ($p<0.01$). ACE2 therapy restored ($p<0.05$) the reduced expression of E-cadherin in both tumour and adjacent tissue, and significantly ($p<0.05$) enhanced CD8+ cytotoxic T-cell infiltration.

Conclusions: Liver-targeted ACE2 gene therapy suppresses both primary and secondary liver cancer progression. We conclude that in HCC, ACE2 promotes the recruitment and activation of innate and adaptive immune cells into the tumour microenvironment, while in secondary liver cancer, ACE2 promotes E-cadherin restoration and CD8+ T-cell recruitment. Thus, liver-targeted ACE2 gene therapy represents a novel therapeutic approach for both primary and secondary liver cancer.

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Oral 9

Developing a Small Animal Model to Use as a Proof of Concept for Therapeutic Splice Modulation of *LDLR* by Antisense Oligonucleotides

Kelly M. Martinovich[1, 2], Will Johnsen[1, 2], Russel Johnsen[1, 2], Steve D. Wilton[1, 2], May T. Aung-Htut[1, 2]

[1] Personalised Medicine Centre, Health Futures Institute, Murdoch University, Murdoch, Perth, WA 6150, Australia.

[2] Perron Institute for Neurological and Translational Science, Nedlands, Perth, WA 6009, Australia.

Background: When developing therapeutic splice-modulating antisense oligonucleotides (ASOs), lead candidate evaluation can only be done in models that contain the deleterious variant, meaning existing animal models cannot be used. Here we phenotype a new mouse model made to reflect a *LDLR* variant that causes Familial hypercholesterolemia. If the phenotype is confirmed, a dermal fibroblast cell line will be established from mice and used to screen ASO sequences before in vivo proof-of-con experiments begin.

Methods: A mouse containing a *ldlr* variant on C57BL/6J background was made by SA Genome Editing Facility. 6-week-old homozygous (*ldlr**/*ldlr**), heterozygous(*ldlr**/*WT)* and wild type (WT/WT) mice were given a high-fat diet (HFD) or control diet (CTRL) for 12 weeks. After 12 weeks on the diet, blood samples were collected for LDL-C measurement and mice were humanely sacrificed, with liver lobes (Lower left lateral and Caudate) and aortas collected and fixed. Tissues were sectioned and stained with Oil Red-O and quantified using QuPath thresholding.

Results: Mice were successfully generated and a colony bred; no physical phenotypic characteristics were noted. Both male and female mice were used in this study. After 12 weeks on a HFD:*ldlr**/*ldlr** mice weighed more than CRTL :*ldlr**/*ldlr**(HFD:*ldlr**/*ldlr**M=40.4g,F=28.3g, CTRL:*ldlr**/*ldlr**M=31.3g,F=22.2g). Blood collected at week 12 showed that only HFD:*ldlr**/*ldlr**mice had elevated LDL-C (28.97mmol/L,p=0.001). HFD:*ldlr**/*ldlr** liver lobe sections had an increase in Oil Red-O staining compared to CTRL:*ldlr**/*ldlr** mice (Left:35%,13%, caudate:36%,13%). Histological sections of the aorta showed evidence of atherosclerosis in 80% HFD:*ldlr**/*ldlr** mice, averaging a 6% lesion area.

Conclusions: This model has key phenotypic characteristics that mimic human condition and other animal models. Personalised animal models are a useful tool that can be used when developing proof-of-concept ASOs and to complement in vitro assays using human-derived samples.

Oral 10

Optimised high rates of gene editing for skin engineering applications using electroporation or a more gentle, yet efficient, cell penetrating peptide

A. du Rand[1], J. Hunt[1], B. Buttle[1], D. Verdon[1], D.J.H.F. Knapp[2], YS Michales[3], R. Dunbar[1], D. Purvis[2], V. Feisst[1] and H.M. Sheppard[1]

[1] School of Biological Sciences, University of Auckland, NZ, and [2]Starship Children's Health, Auckland District Health Board Starship Hospital, Auckland, NZ

[2] Institut de recherche en immunologie et en cancérologie (IRIC) and Département de Pathologie et Biologie Cellulaire, Université de Montréal, QC H3T 1J4, Canada

[3] Paul Albrechtsen Research Institute CancerCare Manitoba, Winnipeg, MB R2H 2A6, Canada.

Background: Conditions that affect the skin are attractive targets for gene editing applications due to the ease of access. We have targeted mutations that cause epidermolysis bullosa (EB), a severe group of monogenic fragile skin conditions, that currently lack a permanent treatment option. We have used gene editing to repair mutations in type VII collagen which cause recessive dystrophic EB, and laminin-332 which cause junctional EB. Using our patented skin-growing techniques these cells can be used to engineer gene-edited, patient-specific skin substitutes *in vitro*, which can be used to permanently cover chronic EB wounds.

Methods: Cell editing was achieved by delivering a formulation of CRISPR/Cas9 ribonucleoproteins (+/- a single stranded oligonucleotide DNA template) by nucleofection, or a simple and gentle mix-and-incubate approach using a cell penetrating peptide (PepFect14-K).

Results: We achieved up to 95% non-homologous end joining (NHEJ)-mediated repair efficiencies, allowing for excision of disease-causing defective exons in the *COL7A1* gene in NZ patient-derived skin cells (primary keratinocytes and fibroblasts).
Not all disease-causing mutations are amenable to an "exon-skipping" approach and instead require homology directed repair (HDR) for precise correction. Targeting the *COL7A1* gene, we achieved Cas9-mediated HDR in up to 75% of patient-derived keratinocytes and >20% in a fibroblast cell population. Using Cas9 nickases, which offer a higher level of specificity, we achieved 60% HDR in the *LAMB3* gene. In each case robust off-target analysis (using nanopore sequencing) did not detect any unintended editing events. We further demonstrated correct gene expression, localisation and functionality by incorporating edited skin cells into lab-grade 3D skin equivalents.

Conclusions: We are now working towards clinical translation by engineering gene edited cells into bilayered skin substitutes using commercially available dermal scaffolds combined with minimal-manipulation techniques. Further experiments are also in progress to assess the feasibility of applying gene editing reagents directly to the skin.

Oral 11

CRISPR-HITI gene therapy for Usher Syndrome Type 1B in patient-derived inner ear and retinal organoids

Vivienne Kaiser[1,2], Nader Aryamanesh[3], Ali Afrasiabi[3], Mark Graham[4], Carmen G. Celemin[5], Elham Nili[1], Simona Rossi[6], Samantha L. Ginn[2,7], Livia S. Carvalho[8], Anai Gonzalez-Cordero[1,2].

[1]*Stem Cell Medicine Unit, Children's Medical Research Institute, Westmead NSW, 2145, Australia*

[2]*Faculty of Medicine and Health, The University of Sydney, Sydney NSW, 2006, Australia*

[3]*Bioinformatics Facility, Children's Medical Research Institute, Westmead NSW, 2145, Australia*

[4]*Biomedical Proteomics Facility, Children's Medical Research Institute, Westmead NSW, 2145, Australia*

[5]*Single Cell Analytics Facility, Children's Medical Research Institute, Westmead NSW, 2145, Australia*

[6]*Ivana Trapani's Group, The Telethon Institute of Genetics and Medicine, Pozzuoli, Italy*

[7]*Gene Therapy Research Unit, Children's Medical Research Institute, Westmead NSW, 2145, Australia*

[8]*Department of Optometry and Vision Sciences, The University of Melbourne, Victoria, 3010, Australia*

Introduction: Usher Syndrome Type 1B (USH1B) causes congenital deafness, early-onset retinitis pigmentosa, and vestibular dysfunction. It arises from mutations in *MYO7A*, which encodes an actin-based motor protein in inner ear hair cells and retinal photoreceptors. USH1B pathology remains poorly understood due to limitations in animal models, and the large size of MYO7A (~7.4 kb) precludes standard AAV approaches. USH1B patient iPSC-derived inner ear (IEO) and retinal organoids (RO) were used to understand pathophysiology and test AAV CRISPR-Homology Independent Targeted Integration (HITI) in a human-context.

Methods: Organoids were generated and analysed by single cell RNA sequencing, mass spectrometry and immunohistochemistry to identify novel disease mechanisms. As organoid transduction has been inefficient in earlier proof-of-concept studies, we first established an improved in vitro AAV transduction method, then tested *MYO7A*-HITI in ROs, assessed by functional assays and omics after therapy.

Results: Organoids proved to be excellent models of disease, with MYO7A absent in both models. RO omics analysis revealed dysregulated phototransduction and actin-related processes. Immunohistochemistry showed a 25% increase in F-actin, a 59% increase in cell body-localised Rhodopsin and 10% outer segment shortening. IEOs displayed elongated, actin-rich stereocilia bundles. HITI-treatment via dual AAV delivery showed Cas9 readily detectable in photoreceptors. After 15 weeks, *MYO7A*-HITI ROs showed ~21% editing, correct donor integration and transgene expression (~1.5%). Bulk methods detected no protein rescue, yet immunohistochemistry showed significant rescue of outer segment and transport defects.

Conclusions: This study established a robust patient-derived organoid platform that revealed novel USH1B disease phenotypes in a human-relevant system. This proof-of-concept CRISPR-HITI system represents a promising tool, overcoming AAV packaging limitations and allowing for the treatment of several patients. Notably, low-level correction leading to restoration of some phenotypes may suggest modest editing may be therapeutically meaningful. We continue to explore these findings to boost editing efficiency and move towards clinical translation.

Oral 12

A Change of Base: CRISPR base editing to correct NF2-SWN point mutations

Alexandra O'Donohue[1,2], Hsien-Yin Kao[1,2], Samantha Ginn[3], Gaetan Burgio[4], Aaron Schindeler[1,2]

[1] Bioengineering and Molecular Medicine Laboratory, The Children's Hospital at Westmead and Westmead Institute for Medical Research, Westmead, NSW 2145, Australia

[2] School of Chemical and Biomolecular Engineering, Faculty of Engineering, The University of Sydney, Sydney, NSW 2006, Australia

[3] Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW 2145, Australia

[4] The John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia

Background / Introduction: Neurofibromatosis-related Schwannomatosis (NF2-SWN) is a complex genetic disorder characterized by nerve-associated tumors. This often affects the auditory nerves unilaterally or bilaterally, leading to hearing loss. Over 30% of NF2-SWN pathological variants are single base substitutions, which could be amenable to repair via CRISPR base editing (CRISPR-BE). This project aimed to demonstrate proof-of-concept for therapeutic correction of a known NF2 patient variant (*NF2* c.169C>T) using a CRISPR-BE approach.

Methods: HEK293T cells were engineered using CRISPR-HDR to be homozygous for the *NF2* c.169C>T variant. Correction was tested using a dual-plasmid approach to express fourth-generation CRISPR base editors (ABE8e, ABEMax, ABE8e-WQ) together with target-specific single guide RNAs. Cells were transfected via Lipfectamine and genomic DNA subsequently analyzed for *NF2* c.169C>T on-target correction and off-target editing (i.e. edits at bystander sites or ranked areas of homology). Editing activity was quantified using targeted amplicon sequencing. In parallel, we generated an *Nf2*$^{169C>T}$ mutant mouse model with locally humanized sequence targetable by the same validated sgRNA guide.

Results: In cells, ABE8e achieved an 84±3.5% on-target correction of *NF2* c.169C>T. Low-frequency bystander editing of c.167T was also observed. ABEMax showed lower editing efficiency and a higher rate of bystander edits. By contrast, ABE8e-WQ reduced bystander editing but achieved less on-target correction (50±4.3%). While off-target editing was detected in 3/10 ranked sites, none were in exonic regions. While heterozygous *Nf2*$^{169C>T/+}$ mice were viable and showed limited baseline penetrance of spontaneous nerve-associated tumors, homozygosity for the *Nf2*$^{169C>T/C>T}$ was incompatible with murine development.

Conclusion: A range of base-editing strategies targeting the *NF2* c.169C>T variant were trialed and could achieve high efficiency *in vitro*. Enzyme engineering (ABE8e-WQ) was able to considerably reduce bystander editing. Current studies are focused on testing this approach with primary murine *Nf2*$^{169C>T/+}$ Schwann cells, with an emphasis on validating glial cell transduction using custom adeno-associated viral vectors.

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Oral 13

A comparative analysis of engineered AAV serotypes to transduce the murine motor unit

Hugo Herron-Vellacott[1], Benjamin Quick[1], Jun Ma[1], Lidia Madrid[2], Heledd Brown-Wright[2], Melissa Reichelt[1], Wally Thomas[1], Shyuan Ngo[1,3], Peter Noakes[1], Adam Walker[2,4], Kelly Walton[1]*, Andrew Tosolini[1,3,5]*Adam Hagg[1]*.

1. School of Biomedical Sciences, The University of Queensland, Brisbane, Australia.
2. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia
3. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia.
4. Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Sydney Australia
5. School of Electrical Engineering and Computer Science, Faculty of Engineering, Architecture and Information Technology, The University of Queensland, Brisbane, Australia.

Background / Introduction: The development of engineered recombinant adeno-associated viral (AAV) capsids with enhanced specificity and efficiency for skeletal muscle and neuromuscular transduction has expanded the toolbox to study muscle biology in the contexts of health and disease. Despite growing use of myotropic and neurotropic AAVs in preclinical and clinical settings, limited direct comparisons between engineered serotypes hinder efforts to identify the most effective vector, information likely to impact fundamental and clinical research.

Methods: Here, we compare the transduction efficiency of engineered myotropic variants (MyoAAV4A and AAVMYO) and ubiquitous versus cell-type-specific promoters, four weeks following muscle-specific and systemic administration via intramuscular or retro-orbital injection, respectively, in adult C57BL/6 mice. Using macro imaging, western blot, and microscopy analyses, we compare reporter protein expression in a series of physiological states.

Results: Consistent with previous reports, both engineered myotropic AAVs displayed efficient transgene expression in skeletal muscle. However, in a direct comparison of intramuscular vector delivery, MyoAAV4A appeared to achieve higher transgene expression, particularly at lower vector genome doses. Neither biological sex nor innervation status of the muscle appeared to alter transgene expression. Expanding our studies beyond the myofibre, we also developed a systemic delivery strategy using myotropic and neurotropic (AAV-PHP.eB) AAVs, enabling efficient and selective targeting of both pre- and post-synaptic compartments of the neuromuscular junction in C57BL/6 mice.

Conclusions: Our findings provide a direct comparison of emerging AAV capsids in skeletal muscle and demonstrate novel strategies for cell-type-specific gene delivery, with potential applications in the contexts of health and disease.

Oral 14

Towards the development of a safe and effective gene therapy for SLC6A1

Rabail Khan[1], Hardik Parate[1], Andrea Perez-Iturralde[1], Simone Borsani[1], Leszek Lisowski[1,2,3]

[1] Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia.

[2] Australian Genome Therapeutics Centre, Children's Medical Research Institute and Sydney Children's Hospitals Network, Westmead, NSW 2145, Australia.

[3] Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine - National Research Institute, Warsaw 04-349, Poland

Background: *SLC6A1*-related neurodevelopmental disorder (SLC6A1-NDD) is a rare genetic disorder characterized by developmental delay and/or intellectual disability, epilepsy, movement disorders, and psychiatric manifestations. It is typically caused by *de novo* loss-of-function variants in *SLC6A1*, which encodes the γ-aminobutyric acid (GABA) transporter 1 (GAT1). Current treatments are largely symptomatic and do not restore GAT1 function. This study aims to develop an AAV-mediated gene therapy strategy to restore *SLC6A1* expression.

Methods: Five endogenous *SLC6A1* promoter variants were evaluated in combination with three AAV capsids: AAV9, PHP.eB, and AAV2.M1. Age-matched male and female C57BL/6 mice received intracerebroventricular administration of the respective vectors. Brains were collected 30 days post-injection and processed for immunohistochemical analysis to assess transgene expression and distribution across brain regions.

Results: Immunohistochemical analysis enabled comparison of transgene expression across the 15 promoter–capsid combinations. The relative efficiency and anatomical distribution of each combination were evaluated to identify vectors capable of achieving robust expression with high specificity in GABA-ergic neurons.

Conclusion: This study will identify an optimized *SLC6A1* promoter–AAV capsid combination for efficient CNS transgene expression. These findings will support the development of an AAV-based gene replacement strategy to restore GAT1 function and provide a foundation for subsequent preclinical evaluation in models of SLC6A1-NDD.

Oral 15

AI-Guided Design of Gene Therapy Vectors: Integrating Cargo and Capsid Modelling

Anne Klein[1], Deborah Nazareth[2], Denis Bauer[3] Leszek Lisowski[4], Laurence Wilson[5]

[1] Australian e-Health Research Centre, Commonwealth Scientific and Industrial Research Organisation, Australia

[2] Translational Vectorology Research Unit, Children's Medical Research Institute, The University of Sydney, Westmead, Australia.

[3] Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Australia; Australia; Macquarie University, Department of Biomedical Sciences, Faculty of Medicine and Health Science, Macquarie Park, Australia.

[4] Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia, Vector and Genome Engineering Facility, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia, Australian Genome Therapeutics Centre, Children's Medical Research Institute and Sydney Children's Hospitals Network, Westmead, Australia, Military Institute of Medicine - *Research Institute, Laboratory of Molecular Oncology and Innovative Therapies, 04-141 Warsaw, Poland*

[5] Australian e-Health Research Centre, Commonwealth Scientific and Industrial Research Organisation, North Ryde, Australia; Macquarie University, Applied BioSciences, Faculty of Science and Engineering, Macquarie Park, Australia.

Gene therapy vector development remains largely dependent on iterative experimental screening, despite vector performance being governed by complex interactions between the therapeutic cargo, capsid structure, packaging constraints, tissue tropism, and host responses. Artificial intelligence and computational modelling provide an opportunity to shift this process toward predictive, mechanism-informed vector design. We are developing an integrated computational framework that addresses both the cargo and vector components of gene delivery. OmniFold combines machine learning with molecular modelling to characterise how nucleic-acid sequence influences folding, compaction, occupied volume, and packaging-relevant properties under spatial confinement. Initially developed for single-stranded DNA, the framework is being extended to RNA, enabling sequence-level optimisation of therapeutic cargos while preserving their encoded function. Complementing this approach, CapBuild provides a computational platform for reconstructing and analysing complete viral capsids from individual structural subunits. The framework enables visualisation and in silico modification of capsid architecture, including residue mapping and structural interrogation of regions associated with tropism, immune recognition, and cargo interactions.

The platform links in with predictive models of vector tropism, allowing researchers to visualise how tissue-specific mutations interact at a structural level. Expansion beyond AAV will enable its application across multiple viral vector systems. Together, these approaches establish the foundation for AI-guided cargo–vector co-design, in which structural, steric and electrostatic properties of both components can be evaluated computationally before experimental testing. Integration with experimental vector-screening datasets will further enable machine-learning models to identify sequence and structural determinants of packaging efficiency, tissue targeting and immune evasion. This strategy aims to move vector engineering from empirical screening toward an iterative design–predict–build–test paradigm, accelerating the development of more efficient and programmable gene delivery systems.

Oral 16

Matrix rigidity amplifies lysosomal dysfunction and fibrotic signalling in Fabry disease iPSC-derived podocytes

Sharon D. Ricardo[1], Jiun-Yan Chiu[1], Ketav Kulkarni[2], Marie-Isabel Aguilar[2], Andrea F. Wise[1], Mark K. Tiong[3], Maria Fuller[4] and Kathleen M. Nicholls[3]

[1] Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

[2]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

[3]Department of Nephrology, The Royal Melbourne Hospital and Department of Medicine (RMH), University of Melbourne, Parkville, Victoria, Australia

[4] SA Pathology and University of Adelaide, Adelaide, South Australia, Australia

Background: Fabry disease (FD) is an X-linked lysosomal disorder in which pathogenic *GLA* variants cause α-galactosidase A (α-Gal A) deficiency and progressive globotriaosylceramide (Gb3) accumulation in podocytes. Emerging gene-, RNA- and cell-based therapies require human models that report variant-specific pathology in a physiologically relevant mechanical context. Conventional podocyte cultures use rigid substrates unlike the glomerular basement membrane, leaving the interplay between matrix rigidity and Gb3 pathology uncharacterised.

Methods: iPSC-derived podocytes were generated from FD patients harbouring p.Met284Thr (*GLA* c.851T>C) and p.Gln398fs (GLA c.1193_1196del) variants, alongside healthy controls, and differentiated over 20 days [1]. Cells were cultured on tunable gelatin-transglutaminase hydrogels spanning 0.5-14 kPa, verified by atomic force microscopy. Readouts included automated high-content immunofluorescence imaging (Opera Phenix), live-cell LAMP-1-GFP imaging, scanning electron microscopy, immunoblotting, AlphaLISA for TGF-β/Smad3 signalling, YAP1, integrin α3 (ITA3) and and label-free mass spectrometry proteomics.

Results: Both variants showed progressive, time-dependent Gb3 accumulation, increased Gb3/LAMP-1 colocalisation and lysosomal enlargement by Day 20, with the greatest Gb3 burden in c.851T>C podocytes. Proteomics identified dysregulated sphingolipid metabolism, reduced α-Gal A and compensatory upregulation of lysosomal hydrolases [2]. Gb3 accumulation per cell increased progressively with substrate stiffness (2-14 kPa), demonstrating a direct association between matrix rigidity and Gb3 burden. In controls, a physiological window (4-10 kPa) optimised viability, arborised morphology and co-upregulation of YAP1 and ITA3, whereas supraphysiological stiffness (13–14 kPa) drove Smad3 hyperactivation, reduced ITA3 and a fibrotic-like phenotype.

Conclusions: Patient-derived iPSC-podocytes recapitulate variant-specific Fabry nephropathy, and matrix rigidity amplifies both Gb3 burden and Smad3-driven fibrotic signalling. This mechanically tunable human platform defines a biomechanically optimal window for podocyte maturation and provides a tractable system for screening gene-, modRNA- and cell-based therapies targeting lysosomal dysfunction in FD.

Support: Shire/Takeda, Sanofi/Genzyme, Royal Melbourne Hospital Fabry Research Fund.

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F. Poster Presentations from Abstracts

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Poster 1

AAVMeter: Element-resolved long-read profiling of intact and off-design rAAV genomes

Ali Afrasiabi[1], Lakshmy Viswanath[2], Ian E Alexander[2,3], Samantha L Ginn[2,3], Nader Aryamanesh[1,3]

[1] Bioinformatics Group, Children's Medical Research Institute, Westmead, NSW, Australia
[2] Gene Therapy Research Unit, Children's Medical Research Institute and Sydney Children's Hospitals Network, Sydney, Australia
[3] Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia

Background: Accurate assessment of recombinant adeno-associated virus (rAAV) vector integrity is critical for gene therapy, because the therapeutically relevant dose depends not only on the number of vector particles administered, but also on the proportion that package intact genomes capable of supporting the intended transgene expression. Long-read sequencing now enables individual rAAVs to be resolved directly; however, established analytical workflows generally emphasize broad structural classifications and do not routinely quantify the completeness of each functional element within individual vector genomes. As a result, they provide limited resolution for determining whether a packaged rAAV contains the full intended genetic architecture. We therefore developed AAVmeter, a modular, element-resolved long-read sequencing pipeline that quantifies the representation of key vector components within individual packaged genomes, providing a sequence-based estimate of the proportion of structurally intact and potentially functional rAAV genomes.

Methods: AAVMeter annotates expected vector elements from the reference construct and maps long reads against a combined AAV, plasmid, and host reference. High-quality AAV reads are then assessed at the individual-molecule level for feature completeness, generating a read-by-feature integrity matrix.

Results: The central output of AAVMeter is a read-by-feature integrity matrix that quantifies how completely each expected component is represented in every individual rAAV read. This provides an element-resolved view of complete, partial, fragmented, and structurally atypical vector genomes. Visualising this matrix reveals changes in element order, orientation, length, and alignment structure, including localised insertions, deletions, and mismatches, enabling alternative rAAV configurations and deviations from the intended design to be identified.

Integrated sequence-origin classification further distinguishes AAV-derived molecules from host- and plasmid-derived sequences, providing a comprehensive view of what is packaged and how closely each genome conforms to the intended construct.

Conclusion: AAVMeter extends long-read rAAV sequencing analysis by providing a construct-aware framework for characterizing intact and off-design vectors, offering a fair proxy for the functional dose.

Poster 2

Highly efficient and cost-effective large gene insertion in primary human T-cells for cancer immunotherapy using advanced genome editing techniques

Benjamin Buttle[1], Daniel Verdon[1], Rod Dunbar[1], Yale Michaels[2], Hilary Sheppard[1]

[1] School of Biological Sciences, University of Auckland, Auckland, New Zealand

[2] University of Manitoba, Winnipeg, Canada

Background / Introduction: Autologous T-cell receptor-engineered (TCR-T) and chimeric antigen receptor (CAR-T) therapies have traditionally relied on retroviral integration of target DNA sequences. This poses the risks of insertional mutagenesis, non-physiological receptor expression and TCR-mispairing, reducing the efficacy of these treatments. Non-viral CRISPR/Cas9 genome editing offers an attractive alternative by eliminating these risks. However, efficient large-insert gene knock-in remains challenging due to low editing efficiencies and homology-directed repair template (HDRT) associated toxicity. We aimed to identify donor template and editing conditions that maximise large gene insertions in primary human T-cells for cancer immunotherapies.

Methods: Primary human T-cells were nucleofected with Cas9 ribonucleoproteins (RNPs) targeting the β-actin (*ACTB*) or T-cell receptor alpha constant (*TRAC*) locus with HDRTs encoding enhanced green fluorescent protein (EGFP) or a TCR sequence. Editing efficiency and T-cell activation were assessed by flow cytometry using EGFP expression or fluorescent pentamer staining following peptide titration assays.

Results: Optimisation of buffer composition, donor template format, and template concentration enabled efficient non-viral insertion of large DNA fragments into primary human T cells. We achieved knock-in of a 1.5 kb EGFP cassette into the *ACTB* locus in up to 53% of cells and a 2.2 kb TCR cassette into the *TRAC* locus in up to 60% of cells. TCR-edited cells maintained high viability (85%) and exhibited activation comparable to a T-cell clone expressing the same receptor.

Conclusions: We developed a highly efficient non-viral CRISPR/Cas9 editing platform for the targeted insertion of large DNA sequences into primary human T-cells. This approach supports generation of functional therapeutic T-cells with high gene insertion rates and viability. This demonstrates a potentially safer alternative to viral vector-based T-cell engineering for cell-based cancer immunotherapies and has potential utility for all large-insert genome editing.

Poster 3

Restoring Nucleocytoplasmic Transport in ALS via ASO-Mediated CHMP7 Knockdown

Caitlyn Vicars [1, 2], Rita Mejzini [1, 2], Frances Theunissen [1, 2, 3, 4], Sophia Luikinga[5], Bradley Turner [1, 6], P. Anthony Akkari [1, 2]

[1] Personalised Medicine Centre, Murdoch University, Murdoch, Australia

[2] Perron Institute for Neurological and Translational Science, Nedlands, Australia

[3] The University of Notre Dame, Fremantle, Australia

[4] King's College London, London, United Kingdom

[5] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

[6] The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Australia

Background / Introduction: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease affecting the motor neurons that control movement. In ~97% of cases, TAR DNA-binding protein 43 (TDP-43) mislocalises to the cytoplasm, disrupting RNA metabolism and neuronal function. Charged multivesicular body protein 7 (CHMP7), a mediator of nuclear pore quality control, abnormally accumulates in neuronal nuclei in ALS, causing nuclear pore injury and contributing to TDP-43 mislocalisation. Previous work shows CHMP7 knockdown prevents and reverses these effects in patient-derived motor neurons, highlighting its promise as a therapeutic target, yet no approved CHMP7-targeting therapy exists. Using a mechanism distinct from existing approaches, we are developing antisense oligonucleotides (ASOs) that reduce CHMP7, hypothesised to restore nuclear pore integrity and TDP-43 localisation.

Methods: Sixteen ASOs were screened in control fibroblasts, with *CHMP7* transcript knockdown assessed by semi-quantitative RT-PCR. Lead sequences will be transfected into ALS patient fibroblasts, with RNA and protein knockdown confirmed by RT-qPCR and western blot, respectively. Optimised ASOs will be transfected into ALS induced pluripotent stem cell (iPSC)-derived motor neurons, where CHMP7 localisation, nuclear pore integrity and TDP-43 nucleocytoplasmic ratio will be assessed by immunofluorescence, cryptic exon inclusion by RT-PCR, and neuronal survival by viability assays.

Results: Lead candidates achieved up to 90% *CHMP7* transcript knockdown in control fibroblasts by RT-PCR densitometry, versus non-targeting control ASO (n=3, one donor line). Building on these findings, optimised ASOs are expected to reduce pathological CHMP7 nuclear accumulation, cryptic exon inclusion, and TDP-43 mislocalisation in ALS iPSC-derived motor neurons, while improving cell viability.

Conclusions: This project will establish whether ASO-mediated CHMP7 knockdown can improve nuclear pore function and resolve TDP-43 dysfunction, providing proof-of-concept for a first-in-class CHMP7-targeting therapeutic. As CHMP7 pathology spans both genetic and sporadic disease, this approach could meet a critical unmet need for the majority of people living with ALS.

Poster 4

National Critical Research Infrastructure for Antisense Therapy Development

May Aung-Htut[1,2], Jessica Cale[1,2], Gareth Baynam[3], Vanessa Fear[4], Ben Dwyer[5], Cara Philpott[1], David Stroud[6], Sue White[7], Jonathan Rodger[8], Wendy Gold[9], Anthony Akkari[1,2], Steve Wilton[1,2],

[1] Personalised Medicine Centre, Health Futures Institute, Murdoch University, Perth, Western Australia

[2] Perron Institute for Neurological and Translational Science, Perth, Western Australia

[3] Rare Care Centre, Perth Children's Hospital, Perth, Western Australia

[4] The Kids Research Institute Australia, Perth, Western Australia

[5] Curtin Medical Research Institut, Curtin University, Perth, Western Australia

[6] Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Victoria, Australia

[7] Murdoch Children's Research Institute, Victoria, Australia

[8] Genetic Health Queensland, Queensland, Australia

[9] Kids Research, the Children's Hospital of Westmead, School of Medical Sciences, the Faculty of Medicine and Health, University of Sydney.

The growing demand for personalised genetic therapies, particularly antisense oligonucleotide (ASO) therapies for individuals with rare and ultra-rare genetic disorders, has highlighted the need for a coordinated national infrastructure to support rapid and scalable therapeutic development. We recently received funding from MRFF to establish a National Research Critical Infrastructure for ASO development in Australia that integrates multidisciplinary expertise and streamlined pre-clinical capabilities.

The facility brings together a nationally distributed network of researchers, clinicians, and consumer representatives with complementary expertise spanning clinical genetics, rare disease care, stem cell biology, proteomics, translational medicine, and ASO therapeutic development. This consortium includes both emerging and established leaders in the field.

The infrastructure will accelerate the development pathway for personalised ASO therapies. At its current stage, the facility focuses on early pre-clinical development, including ASO design, in vitro evaluation, and disease modelling. While toxicology and advanced translational studies are not yet within the scope, expansion to incorporate these capabilities is planned for future development.

This presentation will introduce the facility, outline the services and capabilities currently available to researchers and clinicians, and describe pathways for accessing the infrastructure. We will also discuss the future vision for building a nationally coordinated ecosystem capable of supporting the growing demand for personalised ASO therapeutics and improving outcomes for Australians living with rare genetic diseases.

Poster 5

Targeting DARS1 *In-utero*: AAV Gene Therapy in a Mouse Model of HBSL: AAV Gene Therapy in a Mouse Model of HBSL

Elena Venuti[1], Elizabeth Kalotay[1], Gary D. Housley[1], Matthias Klugmann[1], and Dominik Fröhlich[1]

[1]CNS Gene Therapy group, Translational Neuroscience Facility, School of Biomedical Sciences, The University of New South Wales

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Background: Hypomyelination with Brainstem and Spinal Cord Involvement and Leg Spasticity (HBSL) is caused by DARS1 mutations which impair protein synthesis. The “Massi mouse” (Dars1A274V/D367Y) carries dual patient mutations producing a spectrum of perinatal phenotype severity. This supports prenatal AAV gene therapy as an approach to treat this developmental neurological disorder.

Methods: Massi mice were assessed via echoMRI (body composition), behavioural testing, histology and molecular analyses. Our neuronal-targeting vector, AAV.PHP.eB.CAG-hDARS1, was delivered intraventricularly at gestation day 14–16.

Results: Massi mice show mild-to-moderate symptoms in adulthood, including white matter neuroinflammation. There is markedly reduced pre-weaning survival (~20%) versus the expected Mendelian rate (50%), indicating substantial perinatal lethality. Following prenatal gene therapy, postnatal day 0–2 mortality increased to ~85% of treated litters, compared with ~ 23% reference death rate. The experimentally-induced mortality precluded comparison of Mendelian survival. In surviving animals, transgene expression was confirmed in brain and spinal cord neurons at 6 and 12 months by anti-hDARS1 immunofluorescence; however, no modulation of the phenotype was observed (including maintenance of spinal tract white matter Iba1-positive microgliosis signal).

Conclusion: The Massi model spans a symptom spectrum from perinatal lethality to milder adult phenotypes, making it a translationally relevant model. Despite prenatal intraventricular injections achieving widespread neuronal transduction, high mortality reduced study power. Despite confirmation of strong neuronal DARS1 transgene expression in spinal cord out to 12 months, neuroinflammatory white matter signal persisted, suggesting that a broader pan-neuronal and glial vector approach may be needed.

Poster 6

Streamlining Antisense Therapies for Australian Children with Rare Genetic Diseases

Jessica M. Cale[1,2], Gareth Baynam[3], Vanessa Fear[4,5], Fred Chen[6,7,8], Maina P. Kava[6,9], Gerald F. Watts[8,10], Susan M. White[11,12], Elizabeth E. Palmer[13,14], Emily Oates[13,14], Michelle Farrar[13,14], Steve D. Wilton[1,2], May T. Aung Htut[1,2]

[1] Personalised Medicine Centre, Murdoch University, Perth, Australia

[2] Perron Institute for Neurological and Translational Science, Perth, Australia

[3] Rare Care Centre, Perth Children's Hospital, Perth, Australia

[4] The Kids Research Institute Australia, Perth, Australia

[5] UWA Centre for Child Health Research, Perth, Australia

[6] Perth Children's Hospital, Perth, Australia

[7] Lions Eye Institute, Perth, Australia

[8] Royal Perth Hospital, Perth, Australia

[9] School of Medicine, University of Western Australia, Perth, Australia

[10] University of Western Australia, Perth, Australia

[11] University of Melbourne, Melbourne, Australia

[12] Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Melbourne, Australia

[13] University of New South Wales, Sydney, Australia

[14] Sydney Children's Hospitals Network, Sydney, Australia

Introduction: Despite individually low prevalence, over 7,000 rare diseases collectively affect ~300 million people worldwide. Approximately 80% are genetic in origin and 70% manifest in childhood, accounting for 60% of childhood deaths, with 90% involving significant neurological impairment. Fewer than 5% of rare diseases have an approved treatment. Since 2021, we have been building a platform to streamline personalised antisense oligonucleotide (ASO) therapy development for Australian children with rare genetic diseases, fast-tracking the path from variant discovery to treatment.

Methods: Clinicians and researchers submit variants through our portal for in silico amenability analysis. With informed consent, patient samples (e.g., skin biopsies) are collected. Where appropriate, fibroblasts are used to characterise disease-causing variants and identify candidate ASOs that modulate gene expression. If required, fibroblasts or blood cells can be reprogrammed into induced pluripotent stem cells for more suitable disease models. ASOs can be designed to act through multiple mechanisms and are screened in patient-derived cells for positive changes at the RNA and protein levels. The most effective ASO sequences are optimised and evaluated for functional improvements and in vitro toxicity.

Results: We have cases at every pipeline stage and continue recruiting families through an expanding network of collaborators nationwide. More than 25 submissions have progressed past in silico assessment, with patient-derived samples obtained or underway. Several have completed variant characterisation, confirming pathogenic mechanisms and ASO intervention points. Others are in active ASO development, with candidates under assessment for splice-modulating or expression-correcting potential, and a growing subset has advanced to ASO optimisation and functional assessment.

Conclusions: Rare diseases place a disproportionate burden on affected individuals, families and healthcare systems. Streamlined, personalised ASO development offers a path to faster, more targeted interventions. Collectively, the cases in our pipeline demonstrate that our platform can advance personalised ASO therapies through iterative, data-driven refinement.

Poster 7

Engineering CAR T-cells with improved metabolic fitness and persistence against solid tumour cancers

Joel Lee [1, 2], Isabelle Munoz[1], Paul Beavis[1]

[1] Peter MacCallum Cancer Centre, Melbourne, Australia
[2] University of Melbourne, Parkville, Australia

Introduction: Prior work has demonstrated that CAR T cells with less differentiated, stem-like phenotypes have greater persistence and improved outcomes. Our previous work (Chan et al.2024, *Nature*) showed that FOXO1 overexpression enhances CAR T-cell stemness, persistence, and efficacy. ATAC-seq of FOXO1 CAR T-cells revealed increased chromatin accessibility of transcription factors associated with stemness. This study investigates their role in CAR T-cells.

Methods: NSG mice bearing subcutaneous OVCAR-3 tumours received irradiation (1 Gy), followed by intravenous CAR T-cell infusion (2×10^6) and IL-2 (25K IU,5 daily doses). Tumours were digested for analysis. ATAC-seq motif enrichment was analysed using chromVAR and JASPAR.

Results: Five transcription factors identified from FOXO1 ATAC-seq were evaluated in OVCAR-3 tumour-bearing NSG mice. Gene 1 overexpression significantly increased intratumoral CAR T-cell numbers to levels comparable to FOXO1. Gene 1 CAR T cells showed increased memory markers CD45RA and TCF7 and reduced exhaustion markers TIM3 and CD39, exceeding the effects of FOXO1. Single-cell RNA-seq and ATAC-seq identified unique stem-like $CD8^+$ T-cell clusters induced by FOXO1 and Gene 1, suggesting distinct transcriptional and epigenetic programs.

Mutagenesis was performed to enhance Gene 1 potency. Mutant 1 preserved *in vitro* phenotypes but enhanced *in vivo* anti-tumour activity with 6/6 mice cured by day 30 (controls had 0 cures). At day 9 post-treatment, Mutant 1 increased intratumoral CAR T-cell numbers (~4x more than gene 1) and significantly reduced tumour weights ($p=0.002$ vs the control).

Conclusion: Gene 1 promotes a stem-like CAR T-cell phenotype through distinct transcriptional and epigenetic mechanisms. Mutant 1 greatly enhanced CAR T-cell efficacy and increased intratumoral CAR T-cell numbers. Increased tumour infiltration and reduced exhaustion suggest enhanced persistence and anti-tumour function. The distinct signatures of FOXO1 and Mutant 1 provide a rationale to investigate their co-overexpression for synergistic effects. Overall, Overexpressing Mutant 1 represents a potential strategy for enhancing CAR T-cell efficacy against solid tumours.

Poster 8

Correction of genetic dwarfism (achondroplasia) using CRISPR base editing

Julian Chu[1,2,3], Alexandra O'Donohue[1,3,4], Samantha L Ginn[2,5], Aaron Schindeler[1,3,4]

[1] Bioengineering and Molecular Medicine Laboratory, the Children's Hospital at Westmead, Westmead, NSW, Australia

[2] School of Medical Science, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia

[3] Centre for Diabetes, Obesity and Endocrinology, Westmead Institute of Medical Research, Westmead, NSW, Australia

[4] School of Chemical and Biomolecular Engineering, Faculty of Engineering, The University of Sydney, Sydney, NSW, Australia

[5] Gene Therapy Unit, The Children's Medical Research Institute, Westmead, NSW, Australia

Background: Achondroplasia (ACH) is the most common form of genetic dwarfism. Over 97% of cases are attributed to a recurrent autosomal dominant single-nucleotide variant in the Fibroblast Growth Factor receptor 3 gene (*FGFR3* c.1138G>A). Our team hypothesized that a CRISPR adenine base editor (ABE) approach could be designed to facilitate a corrective, site-specific A→G conversion. An intrinsic limit is efficient delivery to target cells, particularly growth plate chondrocytes. Thus, a secondary goal was to trial chondrotropic adeno-associated virus vectors (AAVs) to find suitable candidates for murine studies and clinical translation.

Methods: HEK293T-ACH cells were engineered by CRISPR-HDR to be homozygous for the *FGFR3* c.1138G>A variant. ABE constructs including nSpRY-ABE8e, SpRY-HF1-ABE8e, and SpRY-HF1-ABE8e^{F148A} were tested using single vector plasmid lipofection. Subsequently, a dual-vector system featuring split-intein reconstitution of ABE systems was rationally designed and also tested by plasmid lipofection. On-target editing, bystander editing, and off-target editing at the top ten predicted sites were quantified by next-generation sequencing. Primary murine epiphyseal chondrocytes were isolated and transduced with a panel of alternatively packaged rAAV-Sox9-tdTomato vectors, and transduction efficiency was assessed by flow cytometry.

Results: HEK293T-ACH cells yielded high-efficiency correction with all SpRY-ABE constructs, with iterative refinement of the enzyme system reducing bystander editing at 1136G>A from 76% to 0%. Moreover, split-intein constructs preserved editing efficiency and are compatible with AAV packaging constraints. Serotype/variant testing of rAAV vectors demonstrated robust transduction with several candidates, with rAAV6.2 (64.7%) being identified as the most promising candidate.

Conclusion: These data demonstrate proof-of-concept for a refined ABE-sgRNA approach able to efficiently correct the ACH variant without significant off-target or bystander editing. Current studies aim to test rAAV6.2 delivery in preclinical cell and mouse models to establish chondrotropic targeting, functional gene correction, and ultimately phenotypic correction.

Poster 9

Human Adrenal Xenografts Reveal Species-Specific AAV Tropism and Enable Vector Selection for *CYP21A2* Genome Editing

Lakshmy Viswanath[1,2], Yuan M. Wang[2,3], David B. Lu[2,3], Sophia H.Y. Liao[4], Leszek Lisowski[2,4], Susan Siew[2,5,6], Stephen Alexander[2,6], Ian E. Alexander[1,2,6], Samantha L. Ginn[1,2,6], and Lara E. Graves[1,2,7]

[1] Gene Therapy Research Unit, Children's Medical Research Institute and Sydney Children's Hospitals Network, Sydney, Australia

[2] Faculty of Medicine and Health, The University of Sydney, Sydney, Australia

[3] Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia

[4] Translational Vectorology Research Unit, Children's Medical Research Institute, Sydney, Australia

[5] Department of Gastroenterology, The Children's Hospital at Westmead, Sydney, Australia

[6] Children's Centre for Transplantation and Research, The Children's Hospital at Westmead, Sydney, Australia

[7] Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, Australia

Congenital adrenal hyperplasia (CAH) is the most common monogenic adrenal disorder. Mutations in *CYP21A2* cause 95% of CAH, making the disease an attractive candidate for gene therapy. We have previously demonstrated proof-of-concept correction of CAH using a dual AAV-mediated gene editing strategy, resulting in targeted integration at the murine *Cyp21a1* locus and partial restoration of adrenal steroidogenesis. However, successful clinical translation requires efficient and selective delivery to human adrenal cells. Species-specific differences in vector tropism may limit the predictive value of conventional mouse models, creating a need for human-relevant platforms. To our knowledge, this represents the first barcoded AAV capsid screen performed in human adrenal xenografts.

Human adrenal tissue obtained from pediatric nephrectomy donors was engrafted beneath the renal capsule of immunodeficient NSG mice. Following a two-week recovery period, mice received a pooled library of 86 uniquely barcoded AAV capsids intravenously. Two weeks after vector administration, mice were harvested. DNA and RNA were isolated, followed by barcode amplification. Next-generation sequencing was performed to quantify capsid presence and compare transduction efficiency across tissues.

A moderate correlation between AAV capsid performance in mouse and human adrenal tissue was found. Several capsids, including AAV9, KP2, and Rh10 showed comparable transduction across both species. In contrast, multiple capsids, including SYD04, AAV11, and RC06, exhibited enhanced transduction in human adrenal tissue despite limited activity in mouse adrenal glands. These findings indicate that murine adrenal tropism does not consistently predict vector performance in human adrenal tissue and may overlook capsids with significant translational potential.

The identification of capsids with preferential transduction of human adrenal tissue supports the use of human-relevant screening approaches to guide vector selection for adrenal-targeted gene therapies. These findings will inform the development of genome editing strategies targeting the human *CYP21A2* locus and accelerate the translation of potentially curative therapies for CAH.

Poster 10

Preclinical development and *in vitro* validation of an AAV-based gene therapy for GATAD2B-Associated Neurodevelopmental Disorder (GAND)

Maddison Knight[1] , Florencia Haase[1] , Matthieu Drouyer[1] , Eboni Elite[1] , Leszek Lisowski[1, 2, 3]

[1] Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia.

[2] Australian Genome Therapeutics Centre, Children's Medical Research Institute and Sydney Children's Hospitals Network, Westmead, NSW 2145, Australia.

[3] Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine - National Research Institute, Warsaw 04-349, Poland.

GATAD2B-associated neurodevelopmental disorder (GAND) is an ultra-rare, predominantly *de novo* disorder primarily caused by heterozygous loss-of-function variants in *GATAD2B*, resulting in haploinsufficiency. *GATAD2B* encodes p66β, a subunit of the nucleosome remodelling and deacetylase (NuRD) complex involved in neurodevelopmental transcriptional programs. Therefore, AAV-mediated gene addition represents a rational therapeutic strategy. However, as p66β is dosage-sensitive, cassette selection must balance sufficient protein restoration while avoiding super-physiological overexpression. This study aimed to develop and evaluate therapeutic transgene cassettes for AAV-mediated gene therapy of GAND using both two- and three-dimensional *in vitro* models.

A panel of fifteen AAV-GATAD2B transgene cassettes was designed, incorporating ubiquitous or predicted endogenous promoter elements, the *GATAD2B* coding sequence, and regulatory elements including 5′ untranslated regions (UTRs), intronic sequences, 3′ UTRs, and polyadenylation elements. Cassette candidates were packaged into AAV2.7m8 and evaluated *in vitro* using *GATAD2B* knockout SH-SY5Y cells, *GATAD2B*-mutant knock-in SH-SY5Y cells and GAND patient-derived cortical brain organoids. *GATAD2B* mRNA and protein expression were assessed by RT-qPCR and western blotting.

Screening across SH-SY5Y cell models identified two cassettes containing ubiquitous promoter elements, as the strongest and most consistent drivers of *GATAD2B* mRNA and protein expression. Additional ubiquitous promoter cassettes demonstrated detectable, but low level, protein expression, whereas cassettes containing predicted endogenous promoter elements displayed limited activity. In contrast, the cassettes containing the endogenous promoter sequences achieved detectable *GATAD2B* mRNA and protein expression in immature patient-derived cortical organoids, suggesting that GATAD2B promoter activity is cell type and developmental stage-dependent. These findings highlight the value of patient-derived organoids for prioritising cassettes that restore *GATAD2B* expression toward physiological levels.

The leading candidate cassettes harbouring either the ubiquitous or endogenous GATAD2B promoter are currently undergoing further evaluation in more mature patient-derived cortical organoids. Collectively, these findings identify promising AAV-GATAD2B therapeutic cassette candidates and establish a framework for their continued preclinical evaluation for GAND.

Poster 11

Reprogramming the liver to prevent diabetes in NOD mice: Evidence for microRNA involvement in hepatic-to-β-cell transdifferentiation

Najah T. Nassif[1], Binhai Ren[1], Jeeva Tomy[1], Dominic O'Sullivan[1], Bronwyn A. O'Brien, Catherine A. Gorrie[1] and Ann M. Simpson[1]

[1]University of Technology Sydney, School of Life Sciences, Sydney, NSW, Australia.

Background: Hepatic-to-pancreatic transdifferentiation, in which liver cells are reprogrammed towards a pancreatic β-cell-like phenotype by delivery of key pancreatic transcription factors (TFs), represents a promising gene therapy approach for type 1 diabetes (T1D). We have previously demonstrated that the third-generation WPT-PNM lentiviral vector, encoding Pdx1, NeuroD1, and MafA prevents hyperglycaemia and maintains glucose-responsive insulin secretion in non-obese diabetic (NOD) mice over a 30-week study period. As key regulators of gene expression, cellular identity, and β-cell function, microRNAs (miRNAs) may provide additional molecular evidence of successful transdifferentiation. This study characterised the expression of selected β-cell-associated miRNAs in WPT-PNM-treated NOD mouse livers.

Methods: Female NOD/Lt mice of 5-6 weeks of age received the WPT-PNM lentiviral vector or saline control. Body weight and blood glucose were monitored until 30 weeks of age. Liver tissues were analysed by RT-PCR and RT-qPCR for expression of pancreatic TFs, hormones (insulin and glucagon), and a panel of candidate miRNAs associated with β-cell maintenance and function.

Results: No WPT-PNM-treated mice developed diabetes by 30 weeks of age. Consistent with previous findings, treated livers expressed a range of endogenous pancreatic transcription factors and pancreatic hormone transcripts. In addition, analysis of the selected miRNAs revealed significant upregulation of □-cell associated miR-375 and miR-26a in transdifferentiated liver tissue, while miR-7, miR-15a, and miR-124 displayed expression changes within the liver tissue consistent with reported roles in β-cell differentiation, maintenance and function.

Conclusions: These findings indicate that WPT-PNM-induced hepatic-to-pancreatic transdifferentiation is accompanied by changes in both coding and non-coding gene expression programs. The observed miRNA expression profile provides additional molecular evidence supporting the establishment of a β-cell-like phenotype in transdifferentiated hepatocytes and highlights the potential of this approach to prevent or delay T1D progression through functional β-cell replacement in the pre-diabetic state.

Poster 12

mRNA therapy restores biochemical imbalances in a mouse model of a Mitochondrial DNA depletion syndrome

Nissa L Carrodus[1], Jenny Yang[2,3], Javier Ramón Pasías[4,5], Keira Turner[2], Yamini Kanse[2], Stavroula Petridi[2,3], Zitian Xiao[2,3], Abhilesh Dhawanjewar[2,3], George Nolan[3], Annelies Quaegebeur[3], Ramon Martí[4,5], Seth W Cheetham[1,6†] and Jelle van den Ameele[2,3†]

[1] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia.

[2] MRC Mitochondrial Biology Unit, University of Cambridge; Cambridge, UK.

[3] Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

[4] Research Group on Neuromuscular and Mitochondrial Diseases, Vall d'Hebron Research Institute (VHIR); Barcelona, Spain

[5] Biomedical Network Research Centre On Rare Diseases (CIBERER), Instituto de Salud Carlos III, Barcelona, Catalonia, Spain

[6] National Biologics Facility, The University of Queensland; Brisbane, Australia.

[†]These authors jointly supervised the work.

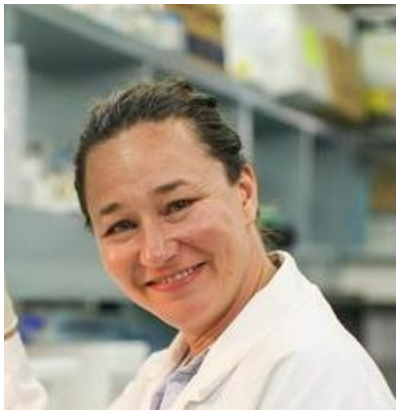
Background / Introduction: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a mitochondrial DNA depletion syndrome (MDS) caused by loss-of-function mutations in the *TYMP* gene which encodes the enzyme thymidine phosphorylase (TP). TP loss leads to systemic elevated levels of the nucleotide precursors thymidine and uridine, leading to mitochondrial nucleotide imbalances and compromised mitochondrial function. Most patients do not survive beyond 40 years of age.
Current treatments transiently restore gene function and biochemical balance via transfusions with erythrocyte-encapsulated TP or platelets, or are highly invasive with a high mortality risk, such as liver transplants. These interventions demonstrate that reinstating TP function to one tissue is sufficient to rescue biochemical imbalance and improve patient symptoms. Amelioration of disease phenotypes in monogenic disorders by delivering mRNA in lipid nanoparticles (LNPs) has been shown, but not in the context of MDS.

Methods: We investigated the delivery of LNPs encapsulating mRNA-encoded *TYMP* to restore nucleoside clearance in a mouse model of MNGIE. Age and sex-matched MNGIE mice were given a single dose of hepatotropic control (eGFP) or TYMP and/or SPAM1 LNPs. Post injection plasma nucleosides were measured via HPLC with TP expression and activity monitored via protein detection and enzyme activity.

Results: Here we show prolonged systemic restoration of the classical MNGIE nucleoside imbalance, via both intravenous and subcutaneous delivery of *TYMP*-mRNA-LNPs, in a mouse model. Injection of *TYMP*-mRNA-LNPs resulted in sustained nucleoside clearance, TP activity and hepatic TP expression for multiple weeks after a single intravenous dose. After 14 days nucleoside levels remained significantly lower than pre-treatment levels and below or within the levels detected in wildtype mice. TP was detected in the liver 21 days after intravenous delivery.

Conclusions: Data presented here will inform dose frequency in a repeat dosing study. Delivery of mRNA-LNPs should be readily translatable to the clinic and could be used to treat other MDS.

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We hope you have enjoyed your time in Melbourne, and we look forward to seeing you again at the 15th AGCTS Biennial meeting in 2028.

