DOI: 10.1002/jgm.3003

WILEY

JOINT 10th AUSTRALASIAN GENE AND CELL THERAPY SOCIETY (AGCTS) AND AUSTRALASIAN SOCIETY FOR STEM CELL RESEARCH (ASSCR) ANNUAL SCIENTIFIC MEETING

Date
May 24 – May 26, 2017
Venue
Aerial Function Centre
The University of Technology, Sydney
Ultimo, New South Wales, Australia

The Australasian Gene and Cell Therapy Society (AGCTS) and the Australasian Society for Stem Cell Research (ASSCR) jointly held their 10th scientific meetings between 24th May and 26th May 2017. The AGCTS and ASSCR acknowledges and thanks the Journal of Gene Medicine for making the abstracts presented during the meeting available to the international research community.

The members of the Organising Committee were:

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For further information, please refer to the AGCTS web page at http://www/agcts.org.au or ASSCR web page at http://www.asscr.org

KEYNOTE PRESENTATIONS

KEYNOTE SPEAKER 1 | LIVER-DIRECTED GENE TRANSFER FOR TRANSPLANTATION TOLERANCE INDUCTION

Alexandra Sharland

Centenary Institute, Australia
Presenting Author: Alexandra Sharland

The liver is a tolerogenic site for the expression of foreign antigens. Liver allografts are spontaneously accepted in both small and large animal experimental models, and up to 20% of clinical liver transplant recipients can be weaned from all immunosuppression. Primary infections of hepatocytes are difficult for the recipient immune response to eradicate and often persist, whilst gene delivery to the liver can induce tolerance to both viral capsid antigens and to the transgene products. Our group uses a gene transfer approach to express allogeneic MHC molecules in the recipient liver, for the induction of transplantation tolerance. High-level expression of donor MHC class I overcomes both naïve and memory responses against skin allografts. Tolerance induction is dependent upon the direct recognition of intact donor MHC. Survival of fully-allogeneic heart grafts is significantly prolonged by the expression of multiple mismatched class I. This work and its potential for clinical translation will be discussed.

KEYNOTE SPEAKER 2 | THE MANY FACES OF AAV: FROM GENE ADDITION TOOL, THROUGH GENE EDITING PLATFORM TO UNEXPLORED EVOLUTIONARY PARTNER OF HUMAN LIVER

Leszek Lisowski[1,2]

[1] *Translational Vectorology Group and Vector and Genome Engineering Facility of the Children's Medical Research Institute, University of Sydney, Sydney, Australia;* [2] *Military Institute of Hygiene and Epidemiology (MIHE), Puławy, Poland*
Presenting Author: Leszek Lisowski

Recombinant viral vectors derived from adeno-associated viruses (AAVs) have emerged as the leading technology for gene transfer in the field of translational virology. More recently, an unprecedented efficiency of AAV-driven genome editing without the need for endonucleases has been reported, opening new avenues for the clinical applicability of this "simple" vector system. This new development has positioned rAAV vectors as an attractive, and potentially safer, alternative to well-established CRISPR and ZFN gene editing tools. It also provides new hope for bridging the gap between currently available strong diagnostic powers and therapeutic benefit / improved health outcomes. However, despite exciting *in vivo* preclinical data from multiple animal models and evidence of early success in the treatment of Haemophilia B, Parkinson's disease and Leber's congenital amaurosis (LCA), clinical data accumulated from over 160 phase I/II/III AAV-based gene therapy trials convincingly demonstrate that current

generation rAAV vectors remains unable to efficiently transduce human cells. Thus, for AAV-based gene therapy to achieve its full potential, novel vectors preselected on human target tissues are needed.

In his talk, Dr. Lisowski will introduce and provide an overview of rAAV selection strategies aiming at identification of novel vector variants with desired clinical properties. The talk will highlight impacts of the selection process and selection system utilized on the function of identified candidates. Dr. Lisowski will describe current efforts undertaken by his group to identify the most recombinogenic AAV variant(s) and the impact of AAV genomic elements on the frequency of genome editing. Finally, the poorly understood, nevertheless evolutionary significant, link between AAV and human hepatocytes will be discussed.

KEYNOTE SPEAKER 3 | THE UPS AND DOWNS OF CF GENE THERAPY

Uta Griesenbach

Imperial Colledge London
Presenting Author: Uta Griesenbach

Although a large number of cystic fibrosis (CF) gene therapy trials have been carried out over the last two decades, these have not addressed whether gene transfer to the lungs can ameliorate CF lung disease. The UK CF Gene Therapy Consortium has developed a programme of both non-viral and lentivirus-mediated gene therapy. The former has identified the optimal plasmid, liposome (pGM169/GL67A) and delivery device. A Phase IIa open label single-dose safety study has identified a safe dose suitable for repeated administration. A double-blind, placebo-controlled multi-dose Phase IIb trial in which subjects received 12 monthly doses of pGM169/GL67A or placebo has been completed. The trial met its primary endpoint and showed, for the first time, that non-viral gene transfer can alter CF lung disease. However, the reponse was comparatively modest and further improvements in gene transfer efficieny are required. To achieve this we have developed a novel lentiviral vector which leads to efficient and long lasting expression in pre-clinical models and have obtained funding for a first-in-man trial.

KEYNOTE SPEAKER 4 | BIOPRINTING LIVING TISSUE

Kimberly A. Homan; David B. Kolesky; Mark A. Skylar-Scott; Jennifer A. Lewis

Wyss Institute, USA
Presenting Author: Kimberly A. Homan

Engineered living tissue constructs could enable new *in vitro* applications in 3D cell studies, drug screening, disease modeling, and, ultimately, therapeutic applications in regenerative medicine. To date, traditional scaffold-based manufacturing processes such as 2D photolithography, bulk casting, gas-forming, and 3D printing are limited in both the scale and complexity of the tissues they can create due to the lack of stable, perfusable 3D vasculature, and the inability to create intricate multicellular configurations and vasculature in 3D. Here, we will detail our recent 3D printing developments enabling the creation of vascular networks and the concurrent patterning of cells and vasculature, along with new strategies for achieving active perfusion, long-term stability, and thicknesses exceeding 1cm, all of which are essential for creating a physiologically and therapeutically relevant tissue manufacturing method. Examples including thick vascularized tissue, stem cell printing, vascular network generation, and renal tissue modeling will all be discussed. With control over multicellular architecture, the chemo-mechanical microenvironment, and the ability to support thick, developing tissue for long time points, this method could serve as a platform for studying emergent biological functions in complex engineered microenvironments, and, may ultimately, find applications *in vivo*.

KEYNOTE SPEAKER 5 | RE-ACTIVATING THE NEONATAL PROLIFERATIVE NETWORK FOR HEART REGENERATION

Enzo R. Porrello[1,2,3]

[1] Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, VIC, Australia; [2] Department of Physiology, The University of Melbourne, Parkville, VIC, Australia; [3] School of Biomedical Sciences, The University of Queensland, St Lucia, QLD, Australia
Presenting Author: Enzo R. Porrello

The inability of the adult mammalian heart to regenerate following injury represents a major barrier in cardiovascular medicine. In contrast, the neonatal mammalian heart retains a transient capacity for regeneration, which is lost shortly after birth. Defining the molecular mechanisms that govern cardiac regenerative capacity in the neonate remains a central goal in regenerative biology. Here, we construct a transcriptional atlas of multiple cardiac cell populations in the neonatal and adult heart, which enables comparative analyses of the response to myocardial infarction for the first time. Our transcriptomic analyses identify a neonatal proliferative network, which is silenced in adult cardiomyocytes and associated with chromatin compaction around cell cycle genes during post-natal maturation. Complimentary high-throughput screening studies in human cardiac organoids revealed that a switch in metabolism from glycolysis to fatty acid oxidation drives post-natal cardiomyocyte cell cycle arrest via repression of β-catenin and Yap-dependent signaling. In ongoing studies, we are employing a number of gene therapy strategies to re-activate the neonatal proliferative network for heart regeneration. These findings uncover key transcriptional circuits underpinning the neonatal regenerative state, which could be harnessed for cardiac regeneration following injury.

KEYNOTE SPEAKER 6 | PRE-MRNA SPLICING AS A THERAPY FOR DUCHENNE MUSCULAR DYSTROPHY

Steve D. Wilton

Centre for Comparative Genomics, Murdoch University, Murdoch, Australia
Presenting Author: Steve D. Wilton

Duchenne muscular dystrophy (DMD), the most common and severe form of childhood muscle wasting, is most frequently caused by frame-shifting

deletions of one or more exons that preclude functional dystrophin expression. Antisense oligonucleotides can redirect pre-mRNA processing so that specific exons are excised from the defective dystrophin mRNA to restore the reading frame and allow expression of a functional dystrophin.

Exondys 51, a phosphorodiamidate morpholino oligomer designed to excise exon 51 from the dystrophin mRNA, is relevant to the most common type of DMD-causing mutation. This compound was granted accelerated approval by the US Food and Drug Administration in September, 2016. *Exondys 51* is the first dystrophin restoring drug, as well as the first specific exon-skipping drug, to be approved for clinical use. Additional phase 3 clinical trials have been initiated evaluating skipping of other dystrophin exons (45 or 53) in order to address different DMD mutations.

Designing clinical trials to evaluate drugs for rare diseases, especially where patient numbers are small and disease progression is slow, poses great challenges. The accelerated approval of Exondys 51 has resulted in vigorous and heated discussion within and outside the FDA, and these issues will be discussed and concerns addressed.

Since the majority of human genes undergo some form of splicing during expression, potential applications for therapeutic intervention during splicing could be relevant to many inherited and acquired conditions. "Therapeutic alternative splicing" is now being developed to a range of conditions, adult onset Pompe's disease to multiple sclerosis, spinal muscular atrophy to cystic fibrosis.

KEYNOTE SPEAKER 7 | THE SYSTEMS BIOLOGY OF STEM CELLS

Christine Wells

University of Melbourne, Australia
Presenting Author: Christine Wells

One of the major goals of systems biology for stem cell researchers is the classification of in vitro derived cells, which may include new stem cell states, or differentiate progeny. Examples include the discovery of 'fuzzy-class' iPSC cells from the Project Grandiose reprogramming study, or assessment of similarity between epiblast and pluripotent stem cells. The Stemformatics.org stem cell atlas is a web platform designed for rapid visualisation and sharing of transcriptome, proteomic and epigenomic data. The resource hosts >8,500 curated samples for public viewing, provides a rapid overview of gene enrichment in different cell types, and hosts a number of easy-to-use visualisation tools to compare groups of genes in individual experiments. Taking advantage of the systematic collation of high quality transcriptome data to mine expression signatures of different cell phenotypes, Stemformatics now hosts the Rohart MSC test. This is an accurate classification tool that can discriminate between mesenchymal stromal cells and related cell types.

KEYNOTE SPEAKER 8 | ROLE OF EPIG ENETIC REGULATORS IN EMBRYONIC STEM CELLS

Partha Das

Monash University, Australia
Presenting Author: Partha Das

Embryonic stem cells (ESC) identity is established through interconnected regulatory networks of transcription factors (TFs), and these TF networks are intimately linked with epigenetic regulators to control chromatin organization and gene expression. However, the role of epigenetic regulators in the networks that establish ESC identity is poorly understood. I would like to talk- the roles of histone demethylases (HDMs), a particular class of epigenetic regulators, in establishing transcriptional regulatory networks for mESCs identity.

In addition, I will also talk- how to discover the potential new epigenetic regulators and regulatory elements (enhancers and superenhancers), and evaluating their functions in mESCs identity, using CRISPR-Cas9 genome editing functional genomics approach.

These will give a broad impression regarding roles of epigenetic regulators in ESCs.

KEYNOTE SPEAKER 9 | ORGANOIDS TO STUDY PANCREATIC CANCER

Christine Chio

Cold Spring Harbour, USA
Presenting Author: Christine Chio

Pancreatic Ductal Adenocarcinoma (PDA) is the 4th leading cause of cancer death in the USA. Lethality of PDA is largely ascribed to poor drug delivery and augmented cell survival pathways. We previously establishment of a new organoid culture system for studying mouse and human pancreatic cancer progression. This model system offers unique advantages given our ability to culture normal pancreatic ductal cells and each stage of the disease from both mouse and human specimens. Using the organoid culture model, we identified proteins involved in glutathione synthesis to be highly up-regulated in premalignant and malignant pancreatic organoids, compared to normal, non-neoplastic counterparts. This suggested redox homeostasis to be a critical biological dependency in PDA. This observation is in line with the finding that oncogenic Kras expression induced an important regulator of redox control, the transcription factor NRF2. Using a platform of human primary organoids, we found that NRF2 was selectively required for the survival of PDA cells, demonstrating a case of synthetic lethality not previously recognized for this transcription factor.

Activation of Nrf2 leads to alterations in cellular redox levels, to which cysteine residues are particularly reactive. Redox modifications on reactive cysteines may regulate the activity of their corresponding protein, rendering these proteins as candidate redox-sensitive effectors of NRF2. To decipher changes in the cysteine proteome, we devised a highly sensitive proteomic method that combines a selectively cleavable cysteine-reactive affinity tag to enrich for and identify reduced cysteines, with amine-reactive isobaric tags for relative and absolute quantification of the total proteome. Using this approach, we identified cysteines on translational regulatory proteins to be explicitly oxidized in Nrf2-deficient, Kras mutant cells. Both cap-dependent and –independent mRNA translation was impaired in Nrf2-deficient pancreatic cancer cells, and can be rescued upon supplementation with antioxidants. In addition to stimulating translation through maintaining the reduced state of specific cysteine residues,

redox regulation by Nrf2 also promotes EGFR autocrine signaling through AKT in KRAS mutant cells to fuel cap-dependent translation initiation. These functions converge to promote global protein synthesis in PDA. As a consequence, combined inhibition of AKT signaling and glutathione synthesis hampered the survival of PDA cells in vitro and in vivo, presenting a new opportunity for therapeutic intervention.

KEYNOTE SPEAKER 10 | EPILEPSY IN A DISH: DISEASE MECHANISM BASED THERAPIES IN NEUROGENETIC DISORDERS

Steven Petrou

Florey Institute of Neuroscience, Department of Medicine (The Royal Melbourne Hospital), The University of Melbourne, VIC, Australia
Presenting Author: Steven Petrou

Epilepsy is a common disease, affecting over 1 in 100 Australians. Those with epilepsy have unpredictable, recurrent seizures that significantly limit their lives. In the most severe forms, known as Epileptic Encephalopathies (EE), the seizures lead to brain injury. Those affected with EE are usually young children so this brain injury leads to impaired physical development, intellectual disability and sometimes death. The causes of EE are varied but genetic causes are common. With the revolution in genetic technology over the last decade, the number of children with EE and a known, causative mutation has very greatly increased. Convergence of this genetic knowledge with disease mechanism studies provides unprecedented opportunity for development of disease mechanism based therapies. The SCN2A gene is amongst the most common cause of epileptic encephalopathy in humans and interesting the single most common cause of de novo genetic autism. Stem cell modelling of patient derived neurons from patients with SCN2A based EE has shown that early and late onset cases are caused by gain and loss of function mutations. These experiments have provided critical clues as to how pathology emerges and converges in these patients but also suggests that for each patient sub-class different therapeutic approaches would be needed. Beyond mechanistic studies stem cell modelling is a key element for the development of gene knockdown based therapies that demand human genome sequence in the appropriate disease state to enable rapid and successful clinical development. As part of this cascade the convergence of advanced gene knockdown technology. electrophysiological, genetic and cell biological approaches is providing new hope for these patients with clinical trials expecting to emerge within 3 years.

KEYNOTE SPEAKER 11 | NOVEL MOLECULAR MECHANISMS WHICH UNDERLIE NEURAL STEM CELL DYSFUNCTION IN HUMAN BRAIN DEVELOPMENTAL DISORDER

Julian Heng

University of Western Australia
Presenting Author: Julian Heng

The development of the mammalian cerebral cortex follows a stepwise production of neurons, then astrocytes and oligodendrocytes from local neural stem cells. Disruptions to stem cell proliferation and neurodifferentiation can lead to human brain developmental disorders including microcephaly, a condition recognised as reduced brain growth during fetal development. We have investigated the molecular mechanisms for microcephaly in Down Syndrome and Intellectual Disability to identify novel cell intrinsic determinants for stem cell development which mediate beta-catenin signalling as well as gene expression regulation. Our approach to model disease-associated genetic mutations improves our understanding of the fundamental mechanisms for brain growth and disease, and supports the improved genetic diagnosis for these neurodevelopmental conditions.

KEYNOTE SPEAKER 12 | ONE YEAR SAFETY AND PATIENT FOCUSED POST HOC EFFICACY ANALYSIS OF GENE THERAPY USING RAAV.SFLT-1 FOR WET AGE RELATED MACULAR DEGENERATION (WET AMD)

Elizabeth Rakoczy

Centre of Ophthalmology and Visual Sciences, The University of Western Australia, Perth, Western Australia
Presenting Author: Elizabeth Rakoczy

Wet AMD is associated with the upregulation of vascular endothelial growth factor, which promotes angiogenesis and vascular permeability. This paper reports on the safety and post hoc analysis of efficacy of rAAV.sFlt-1 which acts like a "biofactory" by producing the endogenous sFLT-1, *in situ* in the eye. Under the protocol, 3 subjects received low dose (LD, 10E10vg), 24 subjects high dose (HD, 10E11vg) rAAV.sFlt-1 and 13 subjects were assigned to a control group. All subjects received ranibizumab (RBZ) injections based on pre-specified criteria for evidence of active wet AMD. During the 1-year follow-up no serious adverse events related to rAAV.sFlt-1 treatment were observed. However, transient adverse events such as subretinal hemorrhage, inflammation, and increased intraocular pressure were reported. LD subjects had improved vision, decrease in retinal center point thickness (CPT) and received an average of 0.33 RBZ injections. Retreatment outcomes in the HD group suggested a bimodal distribution: 14 subjects received ≤2 RBZ (HD1) with improvement in vision accompanied by decrease in CPT. HD subjects receiving ≥3 RBZ retreatments (HD2) had a decrease in vision and a small increase in CPT. Controls averaged 3.5 RBZ injections with decrease in vision and in CPT. 38% controls, 33% LD, 77% HD1 and 20% HD2 had serum antibodies to AAV at baseline. Serum AAV antibodies at baseline did not adversely affect clinical outcomes. Subretinal rAAV.sFlt-1 was well-tolerated with a favorable safety profile in elderly subjects with wet AMD. However, signs of a response with vision gain and fewer RBZ rescue injections were incomplete and suggest the need for further development to optimize delivery and anti-VEGF protein expression.

KEYNOTE SPEAKER 13 | PROGRESS WITH HAEMOPHILIA GENE THERAPY

Amit Nathwani

University College London, UK
Presenting Author: Amit Nathwani

Gene therapy for haemophilia A and B (factor VIII or factor IX deficiency respectively), the most common inherited bleeding disorders, offers the hope of a cure by facilitating continuous endogenous expression of factor VIII or factor IX following transfer of a functional gene to replace the haemophilic patient's own defective gene. Haemophilia may be considered a 'low hanging fruit' for gene therapy because a small increment in blood factor levels (>2% of normal) significantly ameliorates the bleeding diathesis from severe to moderate phenotype, eliminating most spontaneous bleeds. The first trial to provide clear evidence of efficacy after gene transfer in patients with haemophilia B was recently reported by our group. A single peripheral vein infusion of adeno-associated virus (AAV) vector containing the factor IX (FIX) gene led to dose dependent increase in plasma FIX at therapeutic levels with no persistent ill effects. The only toxicity observed was transient subclinical transaminitis at the high dose level, which resolved following corticosteroid treatment. FIX expression has remained stable in most patients for >6years permitting these patients to discontinuation of FIX prophylaxis without increasing the risk of spontaneous haemorrhage. We have not observed any late toxicities. In the last 5 years six new AAV-haemophilia B gene therapy trials have begun with the most promising data emerging from studies using the gain-of-function Padua mutation in the FIX gene. Two studies have report a 6-8-fold enhancement of FIX catalytic activity to 25-80%; levels that are in the normal FIX values. Further advance arse likely to emerge through engineering of capsids to improve the efficiency of AAV gene transfer to the human liver using substantially lower vector doses, thus further improving the safety profile of this vector. This should further improve safety, while easing pressure on. Progress has also been made with haemophilia A, a more challenging target for gene therapy. Using our codon optimised AAV-FVIII expression cassette a BioMarin sponsored study recently showed Factor VIII expression of between 12-150% in 7 severe haemophilia A patients recruited to the high dose cohort. Other gene therapy trial in haemophilia A has commenced and should be reporting soon. Therefore, rapid progress is being made in the field of haemophilia gene therapy. Attention has to now shift on vector production to improve efficiency, quality whilst reducing costs. These and other aspects of haemophilia gene therapy will be discussed.

KEYNOTE SPEAKER 14 | GENE TRANSFER AND TRANSLATION IN NEUROMUSCULAR DISEASE

Jerry R. Mendell[1,2,3]; Samiah Al-Zaidy[1,2]; Richard Shell[2]; W. Dave Arnold[3]; Louise Rodino-Klapac[1,3]; Thomas W. Prior[4]; Carlos Miranda[1]; Linda Lowes[1,2]; Lindsay Alfano[1,2]; Katherine Berry[1,2]; Kathleen Church[1,2]; John T. Kissel[2]; Sukumar Nagendran[5]; James L'Italien[5]; Douglas M. Sproule[5]; Minna Du[5]; Jessica A. Cardenas[5]; Arthur H.M. Burghes[3,6]; K.D. Foust[5]; Kathryn Meyer[1]; Shibi Likhite[1]; Brian K. Kaspar[1,2,3,5]

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Presenting Author: Brian K. Kaspar

Spinal muscular atrophy (SMA) is a devastating, monogenic neurodegenerative disease that in its most severe form, SMA Type 1 (SMA1), afflicted children never sit unassisted, roll over or maintain head control. A natural history study of SMA1 children reported that none achieved a CHOP-INTEND score of ≥40 (with one transient exception at ~41) and 75% died or required permanent-ventilation by 13.6 months. This trial explores safety and efficacy of a single intravenous administration of gene therapy in SMA1. This is the first-ever gene therapy (AVXS-101) trial in SMA1, a rapidly lethal neurologic disease. AVXS-101 delivers the SMN gene in a single-dose via the AAV9 viral vector, which crosses the blood-brain-barrier.

In this ongoing Phase 1 trial, 15 patients with SMA1 confirmed by genetic testing (with 2x*SMN2* copies) were enrolled. Patients received an intravenous dose of AVXS-101 at 6.7e13 vg/kg (Cohort-1 n=3) or 2.0e14vg/kg (Cohort-2, n=12). The primary objective is safety and secondary objectives include survival (avoidance of death/permanent-ventilation) and ability to sit unassisted. CHOP-INTEND scores and motor milestones are additional objectives.

AVXS-101 appears safe and to improve survival (15Sept16 cut-off). All patients are alive and only 1 patient, from Cohort 1, reached the pulmonary endpoint at 28.8 months of age. All patients reaching 13.6 months did so free of permanent ventilation. Patients in Cohort 2 demonstrated improvements in motor function: 11/12 have CHOP-INTEND scores >40 points, 11/12 have head control, and 8/12 sit unassisted. Two patients can crawl, stand and walk independently.

In contrast with the published natural history, a single intravenous administration of AVXS-101 appears to demonstrate a positive impact on the survival of both dosing cohorts and a dramatic, sustained impact on motor function in Cohort-2: 11/12 patients achieved CHOP-INTEND scores and motor milestones rarely or never seen in this population.

KEYNOTE SPEAKER 15 | GUIDED BY EVOLUTION: GENE TRANSFER BIOLOGY AND AAV DISCOVERY

Luk H. Vandenberghe

Grousbeck Gene Therapy Center, Massachusetts Eye and Ear and Harvard medical School, Boston, USA
Presenting Author: Luk H. Vandenberghe

Over the past decades, the *in vivo* gene therapy field has gravitated most of its efforts toward the adeno-associated virus (AAV) for delivery. AAV was repurposed as a potent vector for tissue targeting after an uneventful existence as a naturally occurring and widely prevalent human virus. During its viral evolutionary path, divergent AAV structures arose. Remarkably, when characterized as a vector, it become obvious that even minor changes on its viral capsid, correlated with dramatic altered biological properties relevant to gene therapy. This set of observations has led the field to explore the biological and engineered AAV diversity extensively in order to optimize vector phenotypes such as tissue targeting and specificity, host response and pre-existing immunity, manufacturing and packaging size, etc. The mechanistic understanding however of how capsid changes effectuate these phenotypic changes remains often poorly understood largely because the study of capsid mutations is limited by the architectural constraints of the icosahedral virion structure. Here, we developed a set of strategies and reagents to begin to answer mechanistic aspects of this fascinating and highly relevant structure-function relationship by empirically trying to replay viral evolution through the process of reconstruction of putative ancestral AAV particle structures. This process opened the door to a new approach to AAV capsid discovery, mechanism, and a future of synthetic vector design.

KEYNOTE SPEAKER 16 | THE INDUCED PLURIPOTENCY AND GENOME EDITING REVOLUTION

Sara Howden

Murdoch Childrens Research Institute, Australia
Presenting Author: Sara Howden

The recent development of human induced pluripotent stem cell (iPSC) and gene-editing technologies have fundamentally reshaped our approach to biomedical research, stem cell biology, and human genetics. Reporter lines established in human pluripotent stem cells have been and will continue to be instrumental in facilitating our understanding of human development, drug discovery, toxicology and the development of high-throughput combinatorial screens to facilitate directed differentiation of specific cell types. Enormous research efforts worldwide have also been devoted to disease modeling using iPSCs and it is now becoming more and more apparent that comparisons between gene-corrected and matched isogenic control iPSC lines will be critical for understanding the exact underlying molecular mechanisms governing disease. This talk will highlight some of the previous knowledge that iPSC and gene-editing technologies were built on and discuss some recent developments in this rapidly advancing and exciting area of research.

KEYNOTE SPEAKER 17 | GENE THERAPY FOR CARDIAC ARRHYTHMIAS

Eddy Kizana

Westmead Institute for Medical Research, Australia
Presenting Author: Eddy Kizana

Although gap junction remodelling is widely recognized as a mechanism for ventricular arrhythmias that underpin sudden cardiac death after myocardial infarction (MI), there are no contemporary clinical treatment modalities that target this mechanism directly. We have developed important novel insights into the pathophysiological effects of connexin dys-regulation following MI and exploited these in gene therapy experiments to ameliorate the burden of post-MI ventricular arrhythmia.

KEYNOTE SPEAKER 18 | TRANSPOSON TOOLS FOR CAR T-CELLS

Kenneth Micklethwaite

Westmead Hospital, The Westmead Institute for Medical Research, The University of Sydney, Australia
Presenting Author: Kenneth Micklethwaite

Cell and gene therapy in the form of chimeric antigen receptor T-cells (CAR T-cells) have potential to revolutionise the treatment of a range of malignancies. Challenges impeding the widespread implementation of this technology include cost and complexity of production, an increasingly narrow intellectual property landscape and long term safety concerns. We have developed a simplified protocol for the generation of CAR T-cells using the non-viral PiggyBac transposon system. We have developed in-house CAR constructs targeting CD19 and are proceeding to clinical trials in the setting of relapsed B-cell leukaemia and lymphoma. We are expanding our CAR T-cell repertoire to target other haematological malignancies such as myeloma and acute myeloid leukaemia. We have also developed an alternative transposon system to PiggyBac- called PiggyBat which is free of intellectual property restrictions and we are in the process of assessing in-house safety switches to eliminate CAR T-cells in the case of toxicity. We expect that efforts will enable the widespread application of CAR T-cells for a broad range of malignancies into the future.

KEYNOTE SPEAKER 19 | BIOMATERIALS SUPPORT THE INTEGRATION OF HUMAN EMBRYONIC STEM CELL-DERIVED CORTICAL GRAFTS TO PROMOTE FUNCTIONAL REPAIR IN A MODEL OF FOCAL ISCHEMIA

F.A. Somaa[1]; T.Y. Wang[1]; J.C. Niclis[1]; K.F. Bruggman[2]; J.A. Kauhausen[1]; H. Guo[1]; S. McDougall[1]; R.J. Williams[3]; D.R. Nisbet[2]; L.H. Thompson[1]; C.L. Parish[1]

[1] The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia; [2] Laboratory of Advanced Materials, Research School of Engineering, The Australian National University, Canberra, Australia; [3] School of Engineering, RMIT University, Melbourne, Australia
Presenting Author: C.L. Parish

Stem cell transplants offer significant hope for repairing the brain damaged by ischmic insult. Pre-clinical work suggests that mechanisms underlying therapeutic impact may be multi-faceted – incorporating elements of *bone-fide* circuit reconstruction by transplanted neurons, but also protection and regeneration of host circuitry. In this study we engineered hydrogel scaffolds to form "bio-bridges" within the necrotic lesion cavity to provide physical and trophic support to transplanted human embryonic stem cell-derived cortical progenitors, as well as residual host neurons. Scaffolds were fabricated by the self-assembly of peptides for a laminin-derived epitope, thereby mimicking the brain's major extracellular protein. Following focal ischemia in rats, scaffold-supported cell transplants induced significant and progressive motor improvements over 9 months, compared to Cell- or SAP-only implants. These grafts were larger, exhibited greater cortical differentiation and showed enhanced electrophysiological properties reflective of mature, integrated cortical neurons. Varying the time of grafting after ischemia allowed us to attribute repair to both neuroprotection and circuit replacement. These findings highlight new strategies to improve efficiency of stem cell grafts for brain repair.

KEYNOTE SPEAKER 20 | MUSCLE STEM CELLS IN GROWTH AND REGENERATION

Phong D. Nguyen[1]; Carmen Sonntag[1]; David B. Gurevich[1]; Lucy Hersey[1]; Sara Alaei[1,2]; Hieu T. Nim[1,3]; Ashley Siegel[1]; Thomas E. Hall[1]; Fernando J. Rossello[1,2]; Sarah E. Boyd[1,3]; Jose M. Polo[1,2]; Peter D. Currie[1,4]

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Presenting Author: Peter D. Currie

Organ growth requires a careful balance between cell commitment and stem cell self renewal to maintain tissue growth trajectories. While the processes that regulate resident stem cells during regeneration and disease have received much attention, the basis of stem cell deployment during organ growth remains poorly defined. This knowledge is critical for advancing efforts to generate functioning organ systems *in vitro*. Here we utilize lineage analysis and time lapse imaging to characterize the mechanisms that control stem cell behaviours during skeletal muscle growth in zebrafish. We identify a lifelong stem cell pool that exhibits extensive clonal drift, shifting from the random deployment of a large population of stem cells during larval growth, to the reliance on a small number of dominant stem cell clones to fuel adult muscle growth. We further reveal that self renewal and clonal drift of growth specific muscle stem cells requires the activity of specific genes and cell cycle control. We define a distinct mechanism for the regulation of the stem cells required for global organ growth and in the process provides a molecular understanding of the mechanisms underlying clonal drift *in vivo*.

ORAL PRESENTATIONS

O1 | A COMPARISON OF CLINICAL OUTCOMES BETWEEN TWO OPEN LABEL FDA APPROVED TRIALS OF INTRASPINAL STEM CELL TRANSPLANTATION OF ALS PATIENTS AND AVAILABLE HISTORICAL ALS DATABASES

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We completed a post-hoc analysis of ALS participants enrolled in FDA approved Phase 1 and 2 (Ph1/2) open label studies of intraspinal transplantation of human spinal cord-derived neural stem cells (HSSC) to inform the design of future trials. Ambulatory limb-onset ALS participants (n=22) from Ph1/2 HSSC transplantation trials were compared to limb-onset subjects in two available historical databases: PRO-ACT dataset (n=715) and ceftriaxone study (n=376) with complete data on sex, age, disease duration, and 6 month follow-up. Analyses of survival and a composite survival and functional score (ALS/SURV) was performed against both historical datasets following a matching procedure to participants in the Ph1/2 studies. Survival differed significantly between the Ph1/2 [median survival 4.7years] compared to the matched PRO-ACT cohort [median survival 1.2years] but did not differ significantly between the Ph1/2 [as above] and ceftriaxone matched cohort [median survival 2.3years]. The ALS/SURV, however, revealed that the median participant in the Ph1/2 study had an ALSFRS-R of 19 at 24months (representing 56% of the baseline score), whereas the median participant from both the matched PRO-ACT (p=0.0004) and ceftriaxone (p=0.11) datasets did not live to the 24month visit. In summary, although the HSSC Ph1/2 studies were not designed to assess efficacy, comparison to historical datasets revealed significant improvements in survival relative to the PRO-ACT cohort as well as functional benefits compared to both cohorts. These data support continued evaluation of HSSC transplantation in ALS participants, and enable power calculations necessary to design a future sufficiently powered, randomized, controlled study to evaluate efficacy.

O2 | DEVELOPING CAR T CELLS TO TARGET PAEDIATRIC SOLID TUMOURS

Kenneth Hsu[1]; Shiloh Middlemiss[1]; Radhika Singh[1]; Atul Kamboj[1]; Geoff McCowage[2]; Belinda Kramer[1]

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Despite much needed improvement in the survival of children diagnosed with cancer over the past few decades, improvements have not been achieved across all tumour types. In particular, children with refractory or relapsed solid tumours have a poor prognosis and low survival rates, and there is clear clinical need to develop novel strategies to improve outcomes for these children. Adoptive transfer of gene modified T cells bearing Chimeric Antigen Receptors (CARs) targeting B cell antigens has shown efficacy for treatment of relapsed and refractory leukaemia, pointing to the possibility that CAR therapy may be effective in targeting other tumour types. The aim of this project is to develop CAR T cell therapy for children with relapsed or refractory solid tumours, including osteosarcoma, Ewing's sarcoma, brain tumours and neuroblastoma. The protein, Erythropoietinproducing hepatocellular receptor tyrosine kinase class A2 (EphA2), expressed on a range of paediatric solid tumours and has been identified as a putative target for targeting by CAR T cells. We have tested lentiviral mediated transduced paediatric T cells expressing a second generation CAR targeting EphA2 for cytotoxic activity and activation when incubated with EphA2 expressing osteosarcoma cell lines *in vitro*. Preliminary results show specific and effective T cell mediated cytotoxicity and induction of cytokine secretion using EphA2 bearing tumour cells. We are assembling a panel of other paediatric tumour cell lines, including patient derived primary lines for DIPG, osteosarcoma and glioma to further develop an effective EphA2-redirected CAR T cell therapy with a focus on translating *in vitro* testing into *in vivo* models as a prelude to clinical studies.

O3 | EVALUATION OF EXON SKIPPING USING NOVEL CHEMICALLY-MODIFIED ANTISENSE OLIGONUCLEOTIDES

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Antisense oligonucleotides (AOs) have gained significant interest in recent years towards the development of therapeutics against several diseases. AOs can be used to regulate the function of RNAs by skipping or retaining a specific exon in messenger RNAs (mRNAs) during the RNA splicing process within the nucleus. To improve the pharmacodynamics of AOs, chemical modifications are normally used. Towards this, a number of nucleic acid analogues have been developed in recent years. On September 19th 2016, Eteplirsen, an AO with Phosphorodiamidate morpholino (PMO) chemistry targeting *DMD* exon-51 has been conditionally approved by the US FDA for the treatment of Duchenne muscular dystrophy (DMD). We have investigated the potential of various chemically-modified exon-skipping AOs to induce exon-23 skipping in DMD as model towards improving exon-skipping efficacy. Very recently, we developed a novel analogue of PMO called morpholino nucleic acid (MNA) as the current PMO is not compatible with standard oligonucleotide chemistries, and large-scale production is difficult making PMO very expensive. We investigated the potential of MNA to induce exon-23 skipping in DMD, and found that the MNA-modified AO efficiently induced exon-23 skipping in *mdx* mice myotubes. We also investigated the potential of various other chemistries such as anhydrohexitol nucleic acid (HNA), althreitol nucleic acid (ANA), cyclohexenyl nucleic acid (CeNA), *ortho*-twisted intercalating nucleic acids (*o*TINA) and locked nucleic acids (LNA) to induce exon-23 skipping in DMD, and observed that these modifications can also yield efficient exon-23 skipping.

O4 | DISCOVERY OF A LIVER-SPECIFIC ENHANCER-PROMOTER ELEMENT IN THE 3'UTR OF THE WILD-TYPE AAV2 GENOME PROVIDES NOVEL INSIGHTS INTO AAV VECTOR SAFETY IN THE HUMAN LIVER

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Presenting Author: Ian E. Alexander

Recombinant vectors based on Adeno-Associated Virus (rAAV) have exciting therapeutic potential in the human liver as exemplified by early success in the treatment of haemophilia B. Coupled with ongoing development of capsids with ≥10-fold higher transduction efficiencies than those currently in the clinic, it can be confidently anticipated that many more technically challenging liver diseases will quickly fall within reach of AAV gene transfer technology. While these advances in efficacy are essential, there is an equivalent need to comprehensively address vector safety. A recent report by Nault *et al*., (Nature Genetics, 2015) has drawn an association between AAV2 integration events and hepatocellular carcinoma (HCC) arising in the absence common risk factors. We have independently discovered a novel liver-specific enhancer-promoter element in the 124-nucleotide sequence lying between the AAV2 *cap* stop codon and the right hand inverted terminal repeat. Remarkably this element contains binding sites for known master hepatic transcription factors including HNF1α, HNF4α, HNF6 and GATA6 and is captured in the 163-nucleotide common insertion region of the AAV2 genome integrated into recognized HCC driver genes. While predictively controversial, these finding provide a highly plausible mechanistic link between wide-type AAV2 infection and the rare occurrence of HCC. Once AAV2 proviral carriage rates in the human liver are known, along with integration site preference and proviral structure, we contend that the element we have discovered will prove to be a powerful tool for benchmarking the insertional

mutagenic risk of heterologous enhancer-promoter elements in AAV vectors intended for use in the human liver.

O5 | NOVEL APPROACHES TO PREVENT THE NAASTY – TARGETED GENE THERAPY FOR LEUKODYSTROPHIES

Georg von Jonquieres; Claudia Klugmann; Dominik Fröhlich; Gary D. Housley; Matthias Klugmann

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Presenting Author: Georg von Jonquieres

Recombinant adeno-associated virus (AAV) vectors are versatile and safe tools for CNS gene transfer. Growing evidence suggests that gene replacement therapies are best targeted at the cell type naturally affected by the underlying mutation. Our laboratory has demonstrated that in the rodent CNS glial promoter selectivity can effectively restrict AAV mediated transgene expression to glial cells, even in serotypes that have previously been dubbed 'neurotropic' in the context of non-glial promoters. We have adopted this approach in pre-clinical gene therapies. Canavan disease (CD) is an intractable leukodystrophy with CNS confined pathology. CD is caused by mutations in the gene encoding the oligodendrocyte-specific enzyme aspartoacylase (ASPA) that normally degrades the neuron-derived metabolite N-acetylaspartate (NAA) to acetate and aspartate. Inability to metabolize NAA leads to dramatic and toxic increase of NAA, which is believed to be the root cause of CD. Currently there is no treatment or cure. Using genetic models, we show, that ASPA delivery to oligodendrocytes is paramount to treat CD mice and that post-symptomatic oligodendrocyte-targeted gene replacement is sufficient to revert critical aspects of this vacuolising leukodystrophy. Intracranial delivery of AAV vectors in which *Aspa* is driven by an oligodendroglial promoter reverts damage from NAA toxicity and prevented osmotic consequences, hypomyelination and gliosis thus significantly improving motor performance. In addition our results indicate that creating a metabolic sink in peripheral organs is an effective approach to lower NAA burden and consequently toxicity in the CNS. Our findings are vital with regards to clinical development of novel CD therapies.

O6 | LATE ONSET POMPE DISEASE: RESCUE OF ACID ALPHA-GLUCOSIDASE EXPRESSION BY SPLICE MODIFICATION

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Presenting Author: May Thandar Aung-Htut

A deficiency of the enzyme acid alpha-glucosidase leads to Pompe disease, also known as glycogen storage disease II (GSDII). It is an autosomal recessive disorder, and affected individuals are unable to degrade glycogen stored within lysosomes, leading to an accumulation of glycogen in tissue. Clinically, GSDII may manifest with a broad spectrum of severity, ranging from severe/infantile to a milder late onset adult form. The most common *GAA* mutation associated with the latter form is IVS1-13T>G, found in over two thirds of adult onset GSDII patients. The consequence of this mutation is production of a non-functional *GAA* transcript because exon 2 is excluded during pre-RNA splicing.

Current enzyme replacement therapy for Pompe disease has drawbacks, including immune reaction and poor delivery to target tissues. Therefore an efficient alternative therapy is needed. We sought to restore inclusion of *GAA* exon 2 using splice switching antisense oligomers (AO) directed at splice silencer elements to promote recognition and retention of exon 2 in the mature *GAA* mRNA, thereby restoring enzyme function. Screen of the best AO sequences that promote exon 2 inclusion in *GAA* transcript was carried out in fibroblasts derived from adult Pompe patients. Phosphorodiamidate morpholino oligomers (PMOs) were transfected into patient cells and RT-PCR and acid-alpha-glucosidase enzyme activity were performed. Several PMOs showed an increase in full-length amplicon and increase in GAA activity. These results show that PMO mediated modification of *GAA* transcript could have therapeutic potential for a large portion of adult onset Pompe patients.

O7 | ENHANCED CONVERSION OF ADULT HUMAN FIBROBLASTS INTO INDUCED NEURAL PRECURSOR CELLS USING CHEMICALLY MODIFIED MRNA

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Presenting Author: Bronwen Connor

Direct reprogramming offers a unique approach by which to generate mature neural lineages for the study and treatment of neurological diseases and neurodevelopmental disorders. We have developed an efficient system for directly generating neural stem/precursor cells (iNPs) from adult human fibroblasts by transient ectopic expression of the neural-promoting transcription factors, SOX2 and PAX6. This was achieved using plasmid cDNA which, while desirable in that it reduces potential genomic integration of ectopic factors, results in relatively poor transfection efficiency compared to traditional viral transduction. To advance our direct reprogramming technology, we optimized a chemically modified mRNA gene delivery system (SNIM®) for direct iNP reprogramming. SNIM® RNA has the benefit of being extremely stable and non-immunogenic, allowing us to co-transfect adult human fibroblasts with our reprograming factors SOX2 and PAX6 with an efficiency of >75%, higher than the 10-20% transfection efficiency obtained with plasmids. Cell viability was >70% post-

transfection, significantly greater than 20-40% viability obtained with plasmid transfection. Co-transfection of SOX2 and PAX6 using SNIM® RNA also increases the rate of iNP reprogramming to 14 - 28 days compared to ~45-65 days required using plasmid transfection. The expression of a wide range of neural positional genes were observed through qPCR, and differentiation of SNIM® RNA-derived iNPs generated a mixed neuronal population of a predominantly GABAergic and glutamatergic phenotype. This represent the first time a chemically modified mRNA approach has been used to directly reprogram adult human fibroblasts to iNPs, and provides a rapid system by which to generate human neurons for both research and clinical application.

Funding Source: The Maurice and Phyllis Paykel Trust, the Health Research Council of New Zealand and the New Zealand Tertiary Education Commission.

O8 | TOWARDS A DNA-DIRECTED RNA INTERFERENCE (DDRNAI) THERAPY DELIVERED INTRAVITREALLY USING A NOVEL AAV CAPSID FOR TREATMENT OF AGE-RELATED MACULAR DEGENERATION

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Presenting Author: David Suhy

Background: Inappropriate expression of VEGF growth factors in ocular tissues is implicated in age-related macular degeneration (AMD). We describe a ddRNAi approach to silence VEGF-a, VEGF-b and PGF. Therapeutic evolution of an AAV library in non-human primates (NHP) was used to identify novel capsids showing broad retina transduction following intravitreal injection.

Methods: shRNA inhibition was validated using ARPE19 and JEG3 *in vitro* cell cultures. Therapeutic evolution of an AAV library of up to 10^8 unique AAV capsid variants was performed in NHPs to ensure that novel capsids, delivered via intravitreal injection, traverse the inner limiting membrane. Capsids that could transduce deep retinal layers were recovered and used in subsequent rounds of screening. Two novel capsid variants containing a recombinant GFP construct were tested for biodistribution and tolerability following the intravitreal administration into NHPs and assessed by fluorescence fundus imaging.

Results: The recombinant shRNA construct inhibited angiogenic growth factors expression up to 95% as assessed by protein and mRNA. Inhibition was concomitant with shRNA expression levels. Therapeutic evolution screens revealed several candidate AAV capsids that transduce retinal layers, including the RPE and photoreceptors, following intrvitreal injection. GFP fluorescence demonstrates that a wildtype AAV2 control transduced retina predominantly in the perirfovea. In contrast, one of the novel capsid variants showed strong expression in perifovea, optic nerve head as well as broad distribution across large areas of the mid- and far-periphery of retina.

Conclusions: Collectively, these data provide compelling motivation for the continued development of a ddRNAi based therapeutic to treat AMD.

O9 | OUTCOMES OF A PHASE I TRIAL FOR CANCER GENE THERAPY IN PAEDIATRIC PATIENTS

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Presenting Author: Belinda Kramer

Gene transfer targeting haematopoietic stem cells (HSC) in children has shown sustained therapeutic benefit in genetic diseases affecting immune cell function, and in other genetic diseases such as the leukodystrophies, where mixed success with allogeneic HSC transplantation suggests a possible role for gene correction strategies using autologous cells. The focus of our study was to target the HSC compartment for genetic modification to confer resistance to alkylating chemotherapy, using autologous cells harvested from paediatric patients being treated for a brain tumour with alkylating chemotherapy. In a trial of this strategy in adult glioma patients, significant engraftment of gene modified HSC, expressing the mutant DNA repair protein O6-Methyl-Guanine-Methyl-Transferase (MGMT(P140K)) has shown potential in conferring resistance against the combined effect of O6-Benzylguanine (O6BG)/Temozolomide (TMZ) chemotherapy. Our aim was to test the safety and feasibility of this approach in children with poor prognosis brain tumours. Seven patients received both gene modified HSC and post-infusion O6BG/TMZ chemotherapy, with low level transient engraftment of gene modified cells seen in 4 patients following myelosuppressive conditioning. We were able to demonstrate the safe conduct of a technically complex gene therapy protocol encompassing manufacture of the gene therapy vector, genetically modified cell product and the drug O6BG specifically for the trial, in compliance with local and national regulatory oversight. However, the lack of sustained engraftment of gene modified cells pointed to the difficulties in treating either chemotherapy naïve children with energetic endogenous basal marrow activity, or heavily pre-treated patients with poor pre-infusion bone marrow function.

O10 | AIRWAY STEM CELL TARGETING FOR LUNG GENE THERAPY USING HELPER-DEPENDENT ADENOVIRAL VECTOR

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Presenting Author: Huibi Cao

Direct correction of genetic defects in airway stem cells would have great implications in precision medicine for treating inherited airway diseases, such as Cystic Fibrosis, in light of recent advances in engineering of gene editing tools. However, it is largely unknown whether genes can be efficiently delivered into the airway stem cells. In human, basal cells are the major type of stem cells from large to small airway. In this study, we found abundant basal stem cells in pig trachea, main bronchi and down to small airways showing K5 and K14 positive by immunofluorescent and immunohistochemical staining. More importantly, these basal stem cells in mouse and pig lungs can be targeted by HD-Ad-UBC-LacZ and HD-Ad-CMV-GFP vectors. We have also showed that basal cells from human nasal brushings can be efficiently transduced by HD-Ad-K18-CFTR vector and delivery of the human CFTR gene to these basal cells from CF patients can correct the CFTR channel activity following cell differentiation in air-liquid culture. Our results provide a strong rationale for developing gene therapy approach for achieving permanent airway gene correction.

O11 | HARNESSING INTRONIC DELIVERY OF FUNCTIONAL SHRNAS FOR GENE THERAPY

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Presenting Author: Jim Vadolas

The first human gene therapy trial for β-thalassaemia using lentiviral β-globin gene therapy vectors (LVβ) has recently demonstrated significant clinical efficacy. However, while the LVβ vector was effective in a subset of patients, considerable scope for improvement remains. Sustained β-globin expression and transfusion independence was achieved in patients with non-β^o/β^o-genotypes, whereas in patients with β^o/β^o-genotypes β-globin expression did not reach therapeutic levels. Based on these observations it appears that expression of the β-globin transgene is insufficient. This is likely due to the exclusion of regulatory genetic elements from the β-globin locus, imposed by the size constraints of the gene therapy vector. Instead of increasing transgene expression, data from individuals that co-inherit α- and β-thalassaemia suggest that restoration of globin chain balance through the reduction of α-globin chains can ameliorate the disease phenotype. Here we demonstrate the therapeutic potential of RNAi by combining the utility of the LVβ vector with the intronic delivery of functional short hairpin RNAs (shRNAs). Using this approach we established proof of principle by knockdown of an eGFP reporter in murine erythroid cells. Subsequently, knockdown of human α-globin expression in concert with expression of the β-globin transgene was achieved following transduction of β-thalassaemia patient-derived CD34+ cells, demonstrating the feasibility of this approach to modify α:β-globin ratios for optimal therapeutic benefit. We intend to further explore the use of this vector system to target known modifiers of β-thalassaemia, in combination with β-globin transgene expression, in order to achieve balanced α:β-globin synthesis in patients with β^o/β^o-genotypes.

O12 | WNT REGULATES MUSCLE STEM CELL DIFFERENTIATION REQUIRE B-CATENIN

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Presenting Author: Shuang Cui

Canonical Wnt signalling regulates muscle stem cell/myoblast differentiation, but there have been conflicting reports about the requirement for β-catenin in adult myogenesis. To better understand the role of β-catenin we generated β-catenin null primary adult mouse myoblasts using CRISPR. Null cells had an aberrant rounded morphology and greatly impaired spontaneous- and Wnt3a-induced differentiation. The specific requirement for β-catenin in differentiation was confirmed by rescue experiments using β-catenin transfection. Wildtype myoblasts showed a strong reduction in Pax7 protein expression after Wnt treatment, in contrast β-catenin null cells retained Pax7. The myogenic miRNAs miR133b and miR206 were shown to posttranscriptionally repress Pax7 expression. β-catenin null cells failed to induce miR133b and 206 after Wnt treatment, suggesting that β-catenin is needed to relieve Pax7-mediated inhibition of differentiation.

β-catenin null cells showed delayed induction of key myogenic markers such as myogenin and myosin heavy chain after Wnt treatment, and RNAseq analysis confirmed delayed activation of the global myogenic program. ChIPseq showed that Wnt3a increased MyoD binding at E-box elements in wildtype but not β-catenin null cells. Finally, we identify the membrane fusion protein Myomaker as a novel effector of Wnt signalling in myoblasts. Myomaker was induced by Wnt3a in wildtype but not β-catenin null cells, and analysis of the Myomaker promoter suggests that it is activated by Wnt via MRFs such as MyoD.

In summary we propose that Wnt/β-catenin signalling regulates muscle stem cells differentiation by: 1. Inducing miRNA mediated-Pax7 degradation to relieve Pax7's inhibitory effect on differentiation; 2. Positively regulating pro-differentiation factors including Myomaker via interaction with MyoD.

O13 | EPHA FORWARD SIGNALLINGS ENHANCE HUMAN HAEMATOPOIETIC PROGENITOR CELL MIGRATION AND ADHESION VIA RAC1 ACTIVATION

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Presenting Author: Thao M. Nguyen

Bone marrow stromal cells (BMSCs) regulate the proliferation, differentiation, adhesion and migration of haematopoietic progenitor cells (HSPCs). This study showed that human HSPCs ($CD34^+CD38^-$), but not lineage-committed haematopoietic cell populations, express the tyrosine kinase receptors EphA5 and EphA7. Moreover, we found that the ephrinA5 ligand, the high-affinity binding partner of EphA5 and EphA7, is highly expressed by primary human BMSCs. Previous studies have reported that EphA/ephrinA interactions play important roles in haematopoietic cell trafficking; however, their role in BMSC-support of haematopoiesis is unclear. Herein, we show that stimulating EphA5 and/or EphA7 forward signaling in HSPCs using soluble ephrinA5-Fc molecules promoted human HSPC-derived colony formation significantly and was associated with increased expression of granulocyte macrophage colony-stimulating factor receptor on HSPCs. Studies using functional blocking peptides to EphA5/7 found that disruption of EphA5/ephrinA5 and/or EphA7/ephrinA5 interactions inhibited HSPC function in BMSC-dependent long-term culture-initiating cell assays. Furthermore, the adhesion and migration of HSPCs were increased significantly in the presence of ephrinA5-Fc molecules compared with human immunoglobulin G-treated controls. Conversely, blocking EphA5 activation led to a reduction of HSPC adhesion, whereas inhibiting EphA5 and/or EphA7 activation hindered HSPC migration. Analysis of HSPC cultured in the presence of ephrinA5-Fc showed that EphA forward signaling stimulated Rac1 gene and protein expression, and the Rac1 target molecule WAVE1. Moreover, a significant reduction of ephrinA5-mediated HSPC adhesion and migration was observed in the presence of Rac1 inhibitor. These findings suggest that EphA/ephrinA5 interactions are important in maintaining the HSPC niche mediated in part by activation of Rac1 signaling.

O14 | EXPRESSION OF HUMAN PANCREATIC TRANSCRIPTION FACTORS IN THE LIVERS OF FRG MICE

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Presenting Author: Que T La

Gene therapy is being investigated as a possible cure for Type 1 diabetes (T1D), by engineering artificial β-cells to restore glucose-regulated insulin secretion. We have previously delivered furin-cleavable human insulin (INS-FUR) in a lentiviral vector (HMD) to the livers of streptozotocin-induced-diabetic rats, non-obese-diabetic mice and pancreatectomised pigs with successful amelioration of diabetes and pancreatic transdifferenciation of areas of the liver. The aim of the current study was to test the ability of HMD/INS-FUR to transduce human hepatocytes *in-vivo*, using the FRG mouse model that allows engraftment of human hepatocytes into the livers of the animal. Similar to previous results, the diabetic humanised FRG mice transduced by HMD/INS-FUR (n=4) regained and maintained normal blood glucose levels for 60 days. Their response to an intraperitoneal glucose challenge test was not significantly different from nondiabetic animals. Immunofluorescent microscopy showed the expression of both human insulin and human albumin in the transduced chimeric livers. Human insulin storage was 2.4±0.1nmol in liver isolated from HMD/INS-FUR treated animals and mouse insulin was 0.6±0.1nmol, as compared to 4.4±0.2nmol in normal mouse pancreas. The non-fasting serum of the treated animals had higher levels of human C-peptide (5.61± 0.9 pmol/L) than mouse C-peptide (1.8 ± 0.2pmol/L). RT-PCR of the liver samples obtained from the transduced humanised mice detected the expression of both murine and human pancreatic genes. Our data showed that HDM/INS-FUR can transduce human hepatocytes *in vivo* and that the technology developed by our laboratory can potentially be a cure for T1D in humans.

O15 | THE USE OF ANTISENSE OLIGONUCLEOTIDE-MEDIATED EXON SKIPPING TO TREAT SPINOCEREBELLAR ATAXIA TYPE 3

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Spinocerebellar ataxia type 3 (SCA3) is one of nine polyglutamine disorders. Although SCA3 is pathogenically heterogeneous, the main feature is progressive ataxia, which in turn affects speech, balance and gait of the affected individual. There is currently no cure, nor effective treatment strategy for affected individuals. SCA3 is caused by an expanded polyglutamine tract found in ataxin-3, resulting in conformational changes that lead to toxic gain of function. This expanded glutamine tract is located at the 5' end of the penultimate exon (exon 10) of the *ATXN3* gene (14q32.1).
This study aims to use antisense oligonucleotide (AO) mediated exon skipping to develop a potential therapeutic strategy for the treatment

of SCA3. Preliminary *in vitro* data in healthy cells show that it is possible to create a novel, internally truncated protein, missing the CAG repeat contained in *ATXN3* and still maintain many normal functions. Ongoing research is to be conducted in patient cells to determine whether the CAG expansion will affect the efficacy of the AOs to product a truncated protein. This research shows proof of concept that the removal of exon 10 alone, although out of frame, is possible. Alongside this, another strategy may be to disrupt normal *ATXN3* expression by targeting exons which disrupt the reading frame and thereby down-regulate protein expression. We hypothesize that reducing the normal and mutant protein may alleviate symptoms and/or delay onset or slow progression of the disease.

O16 | RE-TARGETING RAAV6 TOWARDS INFLAMED ENDOTHELIAL CELLS

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Presenting Author: Hannah Pearce

Gene therapy holds great potential for cardiovascular diseases, including atherosclerosis, yet available vectors such as recombinant adeno associated virus (rAAV) transduce the vasculature poorly and have high off target transduction. Studies have shown that targeting agents can re-direct vector tropism, increasing efficacy, and an excellent target for atherosclerosis delivery is vascular cell adhesion molecule (VCAM-1), which is upregulated on inflamed endothelial cells. Therefore, a single-chain antibody (scFv) that binds to VCAM-1 was produced, for site specific and covalent conjugation to the exterior of rAAV6. Using flow cytometry, the scFv could be detected binding specifically to VCAM-1 expressed on endothelial SVEC4-10 and MHEC cells, and was functionalised by Sortase A mediated conjugation to allow biorthogonal click chemistry attachment to azide, without affecting function. The AAV6 was separately functionalised with Methylglyoxal-azide (MGO-N_3) (MGO forms covalent adducts with capsid arginine residues). MGO functionalisation alone removed native tropism, seen by greatly reduced AAV6-GFP transduction into both SVEC4-10 and MHEC cells, and control CHO and HepG2 cells. Arginine residues make up the heparin binding site of AAV6, and heparin inhibited AAV6-GFP transduction, highlighting an underappreciated role for heparin binding in AAV6 transduction. When the anti VCAM-1 scFv was attached through click chemistry (confirmed with western blotting) endothelial, but not control cell, transduction by AAV6-GFP was greatly enhanced, specifically through VCAM-1 binding. Thus this targeting system could have further application in re-directing AAV6 towards inflamed endothelium for therapeutic use. Overall, as all the components are interchangeable, a highly flexible platform technology for gene transfer was established.

O17 | EXPLORING NOVEL THERAPEUTIC CHEMISTRIES IN EXON-SKIPPING FOR DUCHENNE MUSCULAR DYSTROPHY

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Presenting Author: Bao LT

Duchenne muscular dystrophy (DMD) is a muscle wasting, invariably fatal genetic disease caused protein truncating mutations, commonly frame-shifting deletions of one or more exons in the dystrophin gene that abolish functional dystrophin expression. Induced exon-skipping therapy using splice-switching antisense oligonucleotides (AO) has been explored in the treatment of DMD and is currently the only treatment that has been shown to unequivocally slow progression of the disease over several years. In this study, we explored novel nucleic acid chemistries such as anhydrohexitol nucleic acid (HNA), cyclohexenyl nucleic acid (CeNA), altritol nucleic acid (ANA) and Twisted Intercalating Nucleic Acids (TINA) and evaluating their exon-skipping efficacy *in vitro* in the H2K *mdx* mouse cell line. We have designed, synthesised and tested the AOs to induce exon 23 skipping in *Dmd* pre-mRNA. We also characterized critical properties of the AOs such as melting temperature with complementary RNA, stability against enzymatic activity, and cytotoxicity by WST-1 assays. Overall, our results showed that all modified-AOs efficiently induced exon 23 skipping in H2K cells *in vitro*, showed high nuclease resistance and no obvious toxicity. In addition, HNA, CeNA and ANA-modified AOs also limited the unfavourable dual exon 22/23 skipping product. In conclusion, our study highlights the importance of exploring novel antisense chemistries in improving the scope of exon-skipping therapy, and further systematic investigation of locked nucleic acid (LNA)-modified AO-based exon-skipping is currently underway.

O18 | TREATMENT OF GENETIC LIVER DISEASE BY AAV-MEDIATED GENOME EDITING AND SELECTIVE EXPANSION OF REPAIRED HEPATOCYTES

Anais K. Amaya[1]; Cindy Zhu[1]; Samantha L. Ginn[1]; Claus V. Hallwirth[1]; Szun S. Tay[1]; Sharon C. Cunningham[1]; Grant J. Logan[1]; Ian E. Alexander[1,2]

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Presenting Author: Anais K. Amaya

Gene repair involves the correction of a genetic defect directly in the mutant locus. The biggest challenge of this approach, however, is that gene repair by homologous recombination occurs at levels that are unlikely to be sufficient to confer therapeutic benefit in the majority of liver disease phenotypes. To overcome this challenge targeted gene repair approaches have the potential to be complemented by selective expansion strategies designed to increase the number of gene-repaired cells to therapeutically effective thresholds. We are exploiting a selective pharmaco-genetic expansion strategy developed by Grompe and colleagues (Nygaard *et al*., 2016) based on modulation of the tyrosine catabolic pathway. The genetic component of this strategy involves inhibition of hydroxyphenylpyruvate dioxygenase (HPD), a proximal enzyme in this pathway. The aim of this study is to develop the genetic elements required for use in primary human hepatocytes. As an initial step we have successfully identified shRNAs and related miRNAs with potent anti-human HPD mRNA activity *in vitro*. Three miRNA candidates were then selected for *in vivo* screening in Fah$^{-/-}$/Rag2$^{-/-}$/Il2rg$^{-/-}$ (FRG) mice engrafted with primary human hepatocytes. Three weeks after treatment with AAV-miRNA vectors packaged in LK03, a human hepatotropic capsid, livers were harvested for evaluation. The best performing miRNAs achieved ≥90% knockdown of human HPD. The next step will be to test whether these miRNAs confer resistance to CEHPOBA-mediated drug toxicity thereby allowing selective expansion of gene modified hepatocytes. The final step will be to couple this selective pharmaco-genetic expansion strategy with locus-specific gene correction by homologous recombination.

O19 | SELECTION OF NOVEL REPLICATION COMPETENT AAV CAPSID LIBRARIES LEADS TO AN UNEXPECTED OUTCOME IN PRIMARY HUMAN CD34$^+$ AND IPSC-DERIVED NEURON CELLS

Marti Cabanes-Creus[1,4]; Cindy Zhu[2]; Grant Logan[2]; Samantha Ginn[2]; Belinda Kramer[3]; Giorgia Santilli[4]; Adrian Thrasher[4]; Ian E. Alexander[2]; Leszek Lisowski[1,5]

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Presenting Author: Marti Cabanes-Creus

The established ability of the AAV2 vector genome to be cross-packaged into different capsids has enabled the development of high-throughput vector selection strategies based on directed evolution. These strategies rely on the generation of highly-variable AAV capsid libraries and subsequent selection on target cell types, aiming to identify novel candidates with unique properties, such as enhanced transduction. Here we describe the generation of novel, highly-replicative, AAV capsid libraries harboring the 3' UTR sequence of wild-type AAV2, a functionally poorly understood region. This new library underwent replication-based iterative selection in primary human hematopoietic stem cells (CD34$^+$), a cell type highly resistant to AAV transduction. After three rounds of iterative selection, four new AAV serotypes (AAV SYD01-04) were identified, representing 75, 19, 5 and 1% of the selected pool, respectively. Surprisingly, AAV-SYD01 and AAV-SYD02 were also found as the main variants after selection of the same library on iPSC-derived neurons. This unexpected outcome suggests possible tropism towards i) stem-cells, rather than CD34$^+$ cells or neurons and/or ii) an immature, stem cell-like nature of iPSC derived neuronal cells. Notably, selection of the same library on HepG2 cells, an unrelated immortalized hepatic cell line, led to the identification of distinctive AAV variants, suggesting that the selection bias observed with CD34$^+$ cells and neurons was not an intrinsic property of the libraries used, further supporting our hypothesis. While these newly isolated capsids are currently being tested for transduction performance, this work highlights the importance of choosing the appropriate model systems for target-cell specific vector development.

POSTER PRESENTATIONS

P1 | COMPARISON OF CARDIAC MESENCHYMAL STEM CELLS DERIVED FROM ATRIA AND VENTRICLES

Dhanushi Abeygunawardena[1,2]; Elvira Forte[1,3]; Vaibhao Janbandhu[1]; Munira Xaymarden[1,4]; Richard P. Harvey[1]

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Presenting Author: Dhanushi Abeygunawardena

Heart failure due to injured heart muscle is one of the main causes of death worldwide. Cardiac resident progenitor cells hold promise as suitable candidates to be targeted for better injury resolution and cardiac regeneration. Many studies have proposed the atria as a convenient source of progenitor cells for characterization studies as well as for cell therapy approaches. Given the structural and functional differences between atria and ventricles, the main cardiac chambers, we wondered if this could lead to characteristic differences in resident progenitor cells. We focused on the cardiac colony forming unit-fibroblasts (cCFU-Fs) which are a progenitor cell population that gives rise to cardiac mesenchymal stem cells (MSCs) *in vitro*. A comprehensive characterization was carried out to compare atrial and ventricular CFU-Fs in terms of MSC properties and response to an injury situation. Possible niche factors that could lead to functional differences was also investigated. We demonstrate that atria are enriched for CFU-Fs and atrial MSC cultures show a higher growth rate *in vitro*. Atrial CFU-Fs were observed to reside in a relatively more hypoxic environment possibly as a result of lower vascular density in the atrial tissue. Correspondingly, the cells also contained a lower mitochondrial content relative to

ventricular CFU-Fs. Using a myocardial infarction model, we demonstrate that both atrial and ventricular CFU-Fs respond to the injury induced in the left ventricle although in a different manner. In conclusion, this study demonstrates significant functional differences between cCFU-Fs derived from atria and ventricles. This can at least in part be due to the metabolic differences resulting from the relatively hypoxic niche in the atria. Our findings also imply the importance of considering the anatomical location within the heart when using cCFU-Fs and potentially other cardiac resident progenitor cell populations in experimental/ therapeutic approaches.

P2 | MODULATION OF IMMUNE RESPONSES THROUGH COMPLEMENT DOWNREGULATION USING ADENO-ASSOCIATED VIRUS MEDIATED GENE THERAPY

Amina Ahmad[1]; Grant J. Logan[1]; Anna Buchberger[2]; Szun Tay[3]; Peter J. Lachmann[2]; Ian E. Alexander[1,4]

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Presenting Author: Amina Ahmad

This study involves modulation the complement cascade, part of the innate immune system, using adeno-associated virus (AAV) gene delivery to manage inflammatory disorders. AAV are used clinically as gene delivery systems as they can efficiently deliver transgene expression cassettes. The complement system provides protection against pathogens and also clears apoptotic debris, however it can also contribute to the progression of inflammatory disorders. Factor I is a complement regulatory protein and its primary role is to prevent complement activation. We aim to identify the effect of Factor I over-expression on tissue damage and renal function after induced ischemia reperfusion injury in mice, a disease model in which complement activation is a contributing factor. The hypothesis is that increased levels of Factor I in the circulation will downregulate complement activity and reduce injury. AAV encoding Factor I was administered to adult mice and monitored over 8 weeks. Factor I levels in the circulatory system increased five-fold progressively over three weeks and remained stable until the end of the experiment at 8 weeks. Additionally, mice with elevated Factor I exhibited a 50% reduction in complement activity in an in vitro functional assay. However, when subjected to renal ischemia they showed no significant effect on kidney tubular injury or renal function as indicated by creatinine and urea nitrogen levels. Interestingly, female mice did not portray significant tubular injury or elevated creatinine and urea nitrogen levels after renal ischemia. These outcomes have implications for future modulation of immune pathways using gene therapy.

P3 | SINGLE SYNTHETIC DNA REFERENCE MATERIAL FOR MULTIPLE GENE THERAPY VECTORS

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Presenting Author: Anna Baoutina

There is a recognised need for standardisation of protocols for vector genome analysis in vector manufacturing, biodistribution studies, to establish dosage and detect gene doping in sport. Typically such analysis is performed by qPCR using plasmid-based calibrants incorporating transgenic sequences. These often undergo limited characterisation and differ between manufacturers, potentially leading to inaccurate quantification, inconsistent inter-laboratory results and affecting clinical outcomes. Importantly, contamination of negative samples with such calibrants could cause false positive results.

We developed a unique design strategy for synthetic DNA reference materials (RMs) with modified transgenic sequences to prevent false positives due to cross-contamination. Such RMs were amplified in PCR with the same efficiency as a vector; yet, the amplicons from the RM and the transgene were distinguishable based on size and sequence. Using this strategy, we generated a RM for analysis of four human transgenes for IGF1, GH1, GHRH and FST, and characterised it for quantity, purity, homogeneity and stability according to ISO Guide 35. Finally, we validated the RM in analysis of nonviral vectors carrying any of the four transgenes *in vitro* and *ex vivo* in blood using PCR.

The generated RM can be used in gene therapy or detection of doping with one of four genes used in this study. Incorporation of multiple transgenic sequences into one RM reduces the number of RMs needed for analysis of different vectors or transgenes. The design strategy can be used for production of RMs for other transgenes or transcripts for applications requiring standardised, accurate and reliable analysis.

P4 | STUDYING THE BRAIN WITHOUT STUDYING THE BRAIN: 3D NEURAL TISSUE CONSTRUCTS FROM HUMAN IPSCS

Tom Barsby[1,2,4]; Anita Quigley[1,2,3,4]; Justin Bourke[1,2,3]; Jeremy Crook[1,4]; Gordon Wallace[1,4]; Robert Kapsa[1,2,3,4]

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Presenting Author: Tom Barsby

How do we study the human brain? Modern imaging techniques and post mortem studies have taught us a lot about brain structure and function, but more detailed biological and biochemical analysis is impossible in live subjects. Induced Pluripotent Stem Cells (iPSCs) are an incredibly powerful type of stem cell that can generate any cell type

in the human body. Using iPSCs to produce specific brain cells in a dish, it becomes possible to unpick the mechanisms of human brain development and neurological disease outside of someone's body.

This study focuses on the generation of excitatory and inhibitory forebrain neuronal subtypes from human iPSC-derived neural precursor cells (NPCs). The differentiation and maturation of these neural subtypes into functional neural cells form the basis of studying specific regions of human brain development.

We are also developing biomaterial-based hydrogels to aid both neuronal differentiation and network formation within 3D scaffolds. Cell encapsulation in 3D scaffolds allows for a more biomimetic environment for neural development and generates a more physiologically relevant unit of brain biology than planar culture.

The summation of this research is to combine the fields of iPSC technology and tissue engineering to create 3D neural models that shine light on intrinsic pathways and timings of human brain development and how these are perturbed during neurodevelopmental disease pathologies.

P5 | EPIGENETIC ENZYMES AND THEIR EMERGING ROLE IN MESENCHYMAL STEM CELL LINEAGE DETERMINATION

Dimitrios Cakouros[1]; Sarah Hemming[1]; Clara Pribadi[1]; Andrew Zannettino[2]; Stan Gronthos[1]

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Presenting Author: Dimitrios Cakouros

The self renewing properties of stem cells are central to regenerative medicine. The ability to epigenetically modify the stem cell chromatin architecture to improve plasticity, is a key driver in current stem cell research. One of the main impairments to tissue maintenance during ageing is the reduced capacity for stem cell self-renewal over-time due to senescence and impaired differentiation. Histone and DNA modifying enzymes are pivotal to stem cell renewal and lineage determination as shown in studies utilizing embryonic and adult stem cells. Using both functional and transcriptomic approaches we have recently discovered that the interplay between DNA methylation/hydroxymethylation, histone H3 lysine 4,27 and 9 methylation dictates Mesenchymal Stem Cell (MSC) fate. Our work is revealing multiple levels of epigenetic regulation involved in mesenchymal stem cell renewal and how these mechanisms are deregulated in bone related diseases such as osteoporosis and ageing.

P6 | MAGNETICALLY AND PHOTOCHEMICALLY TRIGGERED NANOMATERIAL PLATFORMS FOR NON-VIRAL DNA DELIVERY

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Presenting Author: Wenjie Chen

Effective gene therapy relies on high efficacy of transferring exogenous genes. Liposomes are employed to develop gene and drug delivery systems, widely applied into biomedical fields including gene therapy and chemotherapy. Based on the liposomal delivery platform, we designed two sorts of externally stimuli-triggered liposomes, one encapsulating a clinical photosensitiser (verteporfin, VP) and antisense DNA fragments, and the other complexing with nano magnetic nanoparticles for plasmid DNA (pDNA) delivery. The light-responsive system can be activated by photosensitation, which were employed to silence a specific gene participating in regulations of neurite growth in PC12 cells. Additionally, we demonstrated this light-enhanced manner at subcellular level, particularly carried out the quantitative analysis of colocalization between DNA and endo/lysosomes. Consisting of liposomes and iron oxide nanoparticles, the magnetical liposomal system was utilized to carry double genes into cells efficiently underneath an external magnetic field. The micro morphology were used to evaluate the assembly performance between magnetic liposomal nanoparticles and pDNA molecules. Under observation using atomic force microscopy, the nanoparticle complexes exhibited the compact "fishing-net structure" after combining with pDNA, which would be favourable to increase the gene transfer efficiency due to enhanced condensing and protection of pDNA molecules. These two externally triggered nanocomplexes offered the facile and effective platforms for nonviral gene delivery. By introducing other therapeutic siRNA or DNA, the platforms can be developed to achieve spatiotemporally controllable gene delivery, establishing a photo-assisted or magnetically enhanced system for gene therapy.

P7 | NOVEL MUTATIONS IN TBCD ASSOCIATED WITH SECONDARY MICROCEPHALY

Hayley D. Cullen[1,2*]; Shimon Edvardson[3,6*]; Guoling Tian[4*]; Hannah Vanyai[1,2]; Linh Ngo[1,2]; Saiuj Bhat[1,2]; Adi Aran[5]; Muhannad Daana[6]; Naderah Da'amseh[7]; Bassam Abu-Libdeh[7]; Orly Elpeleg[3#]; Nicholas J. Cowan[4#]; Julian Ik-Tsen Heng[1,2#]

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Presenting Author: Hayley D. Cullen

Mutation in a growing spectrum of genes is known to either cause or contribute to primary or secondary microcephaly. In microcephaly the genetic determinants frequently involve mutations that contribute to or modulate the microtubule cytoskeleton by causing perturbations of neuronal proliferation and migration. Here we describe four patients from two unrelated families each with an infantile neurodegenerative disorder characterized by loss of developmental milestones at 9–24 months of age followed by seizures, dystonia and acquired microcephaly. The patients harboured homozygous missense mutations (A475T and A586V) in TBCD, a gene encoding one of five tubulin-specific chaperones (termed TBCA-E) that function in concert as a nanomachine required for the de novo assembly of the α/β tubulin heterodimer. The latter is the subunit from which microtubule polymers are assembled. We found a reduced intracellular abundance of TBCD in patient fibroblasts to about 10% (in the case of A475T) or 40% (in the case of A586V) compared to age-matched wild type controls. Functional analyses of the mutant proteins revealed a partially compromised ability to participate in the heterodimer assembly pathway. We show via in utero shRNA-mediated suppression that a balanced supply of Tbcd is critical for cortical cell proliferation and radial migration in the developing mouse brain. We conclude that TBCD is a novel functional contributor to the mammalian cerebral cortex development, and that the pathological mechanism resulting from the mutations we describe is likely to involve compromised interactions with one or more TBCD-interacting effectors that influence the dynamics and behaviour of the neuronal cytoskeleton.

P8 | COMPARISON OF HYBRID RECOMBINANT ADENO-ASSOCIATED VIRUS/TRANSPOSON GENE DELIVERY SYSTEMS BASED ON *PIGGYBAC* AND SLEEPING BEAUTY IN THE LIVER

Sharon C. Cunningham[1,2]; Natsuki Sasaki[1]; Cindy Kok[1]; Susan Siew[1]; Claus V. Hallwirth[1]; Nicola A. Hetherington[1]; Andras Nagy[3,4,5]; Ian E. Alexander[1,6]

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Presenting Author: Sharon C Cunningham

Recombinant adeno-associated viral vectors (rAAV) are a highly efficient gene delivery system for liver-targeted gene therapy. It is possible to achieve close to 100% gene modified cells in both newborn and adult mouse livers. However, due to the largely episomal nature of rAAV, the proportion of gene-modified cells decreases dramatically following vector administration to the newborn liver due to the rapid hepatocellular proliferation which accompanies liver growth. To prevent vector loss over time and maintain stable gene expression levels, we have developed a liver-targeted hybrid rAAV/*piggyBac* transposon system to treat metabolic-genetic disease phenotypes with neonatal onset. Using this system, we successfully achieved stable therapeutic transgene expression in mouse models of urea cycle defects and PFIC3. An alternative transposon system, Sleeping Beauty (SB), has been widely used to gene-modify T cells and generate iPSCs, however to date no studies have reported using a hybrid rAAV/SB transposon system in the liver. We therefore set out to compare *piggyBac* and SB (SB11, SB100) in the context of a rAAV hybrid system. Both systems contained an eGFP expression cassette flanked by transposase recognition sites, and vectors were pseudoserotyped with the AAV8 capsid. Vectors were delivered to newborn mice at similar doses (GFP transposon, $1x10^{11}$ vg/mouse: transposase, $5x10^{10}$ vg/mouse), and livers analysed 4 weeks post-injection. Using FACS analysis, the percentage of gene-modified cells using the *piggyBac* system (99.4±0.1%) was significantly higher than with SB11 (16.8±4.3%) and SB100 (68.7±10.7%). The mean fluorescent intensity was also significantly higher using the *piggyBac* system. We conclude that the hybrid rAAV/*piggyBac* system is superior to Sleeping Beauty in the murine liver.

P9 | MESENCHYMAL STEM CELL DIFFERENTIATION IS MODULATED BY COMPLEX SUGARS IN MPS DISEASE

Sharon Byers[1,2,3]; Nathan Rout-Pitt[1,2]; Hannah Linard[1,3]; Srimayee Vaidyanathan[1,3]; Ainslie L.K. Derrick-Roberts[1,2]

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Presenting Author: Ainslie L.K. Derrick-Roberts

The mucopolysaccharidoses (MPS) are a group of inborn errors of metabolism resulting from the impaired intracellular degradation of complex sugars (glycosaminoglycans, GAGs). Undegraded and partially degraded GAGs build-up within cells, in the circulation and increased levels are excreted in urine. Mesenchymal stem cells give rise to many of the cell types affected by MPS and their differentiation is known to be regulated by specific GAG structures. In this study the effect of exogenous MPS GAGs on mesenchymal stem cell differentiation into osteoblasts, chondrocytes and adipocytes was assessed *in vitro*.

GAGs were isolated from the urine of MPS I (HS and DS GAG), MPS II (HS and DS GAG), MPS IVA (KS GAG) and MPS VI (DS GAG) patients. Human mesenchymal stem cells were incubated with 0 – 5 g/mL of MPS urinary GAG and induced to differentiate by the addition of osteogenic, adipogenic and chondrogenic media.

MPS II, MPS IVA and MPS VI GAGs significantly inhibited both osteogenesis and adipogenesis in a dose dependent manner. MPS I GAG decreased osteogenesis but not significantly. In contrast, control HS, KS and DS GAGs purchased from commercial sources increased both osteogenesis and adipogenesis. MPS IVA and MPS VI GAG decreased chondrogenesis but MPS I and MPS II GAG had no effect. Further work is required to understand the GAG structures responsible for modulating mesenchymal stem cell differentiation but these specific structures that accumulate in MPS can have a dramatic effect on modulating stem cell differentiation *in vitro*.

P10 | EVALUATION OF TUMOR SPECIFIC PROMOTERS FOR TRANSCRIPTIONAL TARGETING OF HEPATOCELLULAR CARCINOMA

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Presenting Author: Bijay Dhungel

Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer and represents the third most common cause of death from cancer. Limited therapeutic options, especially in an advanced stage, combined with the presence of underlying liver dysfunction in most of the patients limit its treatment. Targeted gene therapy may be a promising treatment in this setting. Transcriptional targeting of cancer can be achieved using promoters preferentially active in tumor cells (tumor specific promoters (TSPs)). Alpha Fetoprotein (AFP) and Glypican 3 (GPC3) are oncofetal proteins highly expressed in HCC and not in normal or cirrhotic liver. Given the HCC-specific nature of their expression, we hypothesized that the promoters for these genes should be preferentially active in HCC.

To test this hypothesis we evaluated the potential of the AFP and GPC3 promoters for transcriptional targeting of HCC. The activity of these promoters in a panel of normal liver, HCC and non-HCC cell lines was evaluated by correlating it with the expression levels of corresponding genes using real time PCR. AFP promoter was obtained from the pGL3-AFP plasmid and the GPC3 promoter was isolated from HCC cells. Both the TSPs and the ubiquitous cytomegalovirus (CMV) promoter were cloned into an expression vector to drive the expression of enhanced yellow fluorescence protein (eYFP). The expression level of eYFP for each cell type was quantified using the flow cytometer and normalized against the CMV promoter. Furthermore, transcriptionally targeted suicide gene therapy for HCC cell lines was demonstrated using cytosine deaminase (CD). Cell death assay was performed using Annexin V staining and MTS assay was used to study the percentage of proliferating cells. Similar to the eYFP reporter expression, the percentage of cell death and proliferation was normalized to the CMV promoter.

GPC3 and AFP were detected in 5 out of 7 HCC cell lines studied. This was in line with human patient tumours where the expression has been reported in up to 50-80% of cases. In contrast, the expression was lacking in normal liver and most other non-HCC tumor cell lines. Interestingly a number of non-HCC cell lines showed significant levels of AFP and GPC3 expression. Next, we assessed whether the TSPs could limit transgene expression to HCC. We found that the promoters were able to preferentially induce the expression of eYFP in HCC cells when compared to normal liver cells. While the GPC3 promoter appeared to allow selective expression in HCC compared to normal liver cells, we did detect GPC3 driven transgene expression in some non-HCC cancer cells, which was also seen with the AFP promoter. Additionally, we demonstrate that the expression of suicide gene can be limited to HCC cells compared to the normal liver cells. In conclusion, although transcriptional targeting of transgene expression directs expression away from normal liver cells to HCC, it may require other targeting strategies such as transductional targeting (when using delivery vectors) to achieve higher levels of targeted cancer gene therapy.

P11 | HOW DO WE KNOW IF CYSTIC FIBROSIS LUNG GENE THERAPY WORKS? THE IMPORTANCE OF THE RIGHT ANIMAL MODEL, AND THE RIGHT MEASUREMENT METHOD

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Presenting Author: Martin Donnelley

The development of an efficient gene therapy for cystic fibrosis (CF) lung disease has been constrained by a) the lack of a good small animal model with human-like CF lung disease, and b) the ability to rapidly and accurately pinpoint and measure changes in local lung disease.

CF mice do not exhibit human-like CF lung pathophysiology. In 2016 we employed CRISPR-Cas9 gene-editing to establish a CF rat colony in Adelaide, with the first CF animals available for study by mid-2017. This colony will mean that, for the first time, we can rapidly and routinely perform CF lung gene therapy studies to provide disease-specific data for translation to potential human use.

The severity of CF lung disease is normally measured using lung function tests that produce essentially a single measurement of how the whole lung is functioning. They have limited ability to detect early disease and small changes in lung function, or to identify their origin within the lung. We have been critical collaborators in the development of a new

application of X-ray imaging that gathers information on lung motion, rather than structure, during normal breathing. With advanced processing techniques this gives us visually rich and quantifiable information on lung function in any airway or region in the lung, allowing us to detect, quantify and follow changes in regional lung function over time.

The development of these tools to help validate the efficacy of our HIV-1 VSV-G based CFTR lentiviral gene vector for preventing or halting CF airway disease will be examined.

P12 | HIGH THROUGHPUT SINGLE-CELL MRNA SEQUENCING REVEALS CELLULAR HETEROGENEITY IN CARDIAC MSCS AFTER MYOCARDIAL INFARCTION (MI)

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Presenting Author: Nona Farbehi

The field of transcriptomics has mainly focused on bulk-level measurements that mask significant differences between individual cells. With the advent of single-cell RNA-Seq, it is possible to profile the responses of individual cells at unprecedented depth and thereby uncover, transcriptome-wide, heterogeneity that exists within these populations.

The adult heart contains a population of MSC-like colony forming stromal cells with the cell surface profile $SCA1^{+}/PDGFR\alpha^{+}/CD31^{-}$, termed cardiac colony-forming units-fibroblasts (cCFU-F), as first described by Chong et al. (Chong et al., 2011). The identification of cCFU-F as stem cells is based on their colony formation ability, long-term self-renewal and lineage plasticity *in vitro*. It is hypothesized that cCFU-F cells and their descendant stromal cells are recruited for cardiac repair following myocardial infarction. In a mouse with a H2B-eGFP fusion gene knocked-in to the *Pdgfra* locus, cCFU-F were enriched in the GFP^{high} population. After MI, a GFP^{med} population appears, which has partially lost the expression of SCA1 and PDGFRα proteins, and consists largely of activated myofibroblasts.

The aim of this study was to perform single cell RNA-Seq analysis to dissect heterogeneity within the cCFU-F and stromal compartments of the cardiac interstitium, and to investigate the transcriptome changes in these cells as they shift from GFP^{high} to GFP^{med} cells after MI.

An automated microfluidic platform (10 X Chromium systems) was used to capture and lyse individual $GFP^{+}/PECAM\text{-}1^{-}$ cells (~2000 single cells) of sham (control) and MI mice, reverse transcribe RNA and amplify cDNA. RNA-seq libraries from the amplification products of single cells were sequenced using the Illumina HiSeq2500 platform. Principle component and cluster analysis will be presented for the $GFP^{+}/PECAM\text{-}1^{-}$ cellular compartment.

Chong, J. J. (2011) *Cell Stem Cell*, *9*(6), 527-540. doi:10.1016/j.stem.2011.10.002

P13 | ANALYSIS OF ADENO-ASSOCIATED VIRAL VECTOR SHEDDING FOLLOWING CARDIAC DELIVERY IN A LARGE ANIMAL MODEL

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Presenting Author: M Farraha

Introduction: The risk of dissemination of a viral vector into the environment via excreta from a patient treated with recombinant adeno-associated viral (AAV) vectors – a phenomenon called shedding – is a potential biosafety concern. Despite the number of gene therapy studies undertaken to date, there is no cohesive shedding data in a large animal model.

Aim: To investigate the shedding profile of recombinant adeno-associated viral (rAAV) vectors following intracoronary delivery in a sheep model.

Methods and Results: Sheep in this study were randomised to receive up to 10^{13} vector genomes of rAAV2/6, rAAV2/8 or rAAV2/9 expressing a green fluorescent protein (GFP) delivered via the intracoronary route. Sheep were survived and monitored for 4-days post infusion for shedding analysis. Urine, faeces, mucus and saliva samples were collected before vector infusion and twice daily after infusion. Following sample processing, quantitative real time PCR (qPCR) and functionality assays were performed. qPCR analysis was performed on specimens collected from 6 sheep. The results showed no detectable vector particles in the mucus at all time points. There are no detectable vector particles in the urine after 24 hours. And there are minimally detectable vector particles in the saliva and faeces up to day 4 post infusion. However, further analysis did not reveal functional vector particles.

Conclusion: We conclude that there is no shedding of functional vector particles during the four-day window. This indicates that shedding of rAAV particles poses minimal biosafety hazards.

P14 | HUMAN NEURAL STEM CELLS IMPROVE COGNITION AND DISEASE PATHOLOGY IN THE APP/PS1 ALZHEIMER's MOUSE MODEL

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Presenting Author: Eva L. Feldman M.D., Ph.D.

Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disorder, with no effective treatments. Cellular therapies have the potential to impact the complex AD pathologies by multiple mechanisms. We developed a novel neural stem cell (NSC) line that produces multiple neuroprotective growth factors, facilitating direct, sustained environmental enrichment in target areas of the brain. The goal of this study was to determine the therapeutic impact of NSC transplantation in a mouse model of AD. Human NSCs or vehicle (sham) were administered bilaterally to the peri-hippocampal area of male transgenic APP/PS1 mice. All animals received immunosuppression until the study endpoint. Animals were subject to two hippocampal-dependent behavioral tasks: novel object recognition (NOR) and Morris Water Maze (MWM). AD pathology and grafted NSC survival were assessed using standard immunohistochemistry. NSC transplantation preserved short-term non-associative memory 4wk post-transplant in the NOR test. In the MWM at 16wk post-transplant, NSC-treated animals exhibited improved spatial reference and working memory, indicated by significantly decreased latencies and a strong memory for the former platform location relative to controls. At the study endpoint, amyloid plaque load was significantly reduced within the cortex and hippocampus of NSC-treated animals. Interestingly, we also identified a significant increase in IBA-1 positive microglia exhibiting activated morphologies in these brain areas, suggesting a potential immunomodulatory role of NSCs *in vivo*. In summary, peri-hippocampal transplantation of human NSCs impacts behavioral and pathological phenotypes in the APP/PS1 mouse, supporting testing in additional models and further development as a potential disease modifying therapy for AD patients.

P15 | MULTIPLE EXON SKIPPING TO CORRECT DUPLICATIONS IN THE DYSTROPHIN GENE

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Presenting Author: Kane Greer

Duchenne Muscular Dystrophy is a severe muscle wasting disease caused by protein truncating mutations in *DMD* that results in the absence of a functional protein. Exonic deletions are the most common type of *DMD* lesion, however whole exon duplications account for approximately 10-15% of all reported mutations. Here we describe *in vitro* evaluation of two different antisense oligomer chemistries to correct exonic duplications of the *DMD* gene through splice switching. Phosphorodiamidate morpholino oligomers coupled to a cell penetrating peptide and 2'O methyl phosphorothioate antisense oligomers were tested on four Duchenne muscular dystrophy patient cell strains with exon duplications in the actin binding domain. In all patient cell strains, the removal of exons 6-8 was needed in order to generate an in-frame transcript. Two PCR systems were compared, one using a DNA polymerase designed for sensitivity, specificity and yield (SSIII/AmpliTaq Gold) and one using a high fidelity PCR enzyme for long range PCR (Takara LA Taq). Apparent differences in exon skipping efficiencies were evident. Takara LA Taq produced more of the full length duplicated transcript in comparison to the SSIII/AmpliTaq Gold amplification system. Due to incurred "PCR slippage", the SSIII/Amplitaq Gold system resulted in preferential amplification of the smaller PCR products, as well as substantial amounts of an apparently normal dystrophin transcript. Combinations of phosphorodiamidate morpholino oligomers targeting exons 6-8 in the patient cell strains were more efficient at restoring a multi- exon skipped in-frame transcript compared to the same combinations of oligomers with the 2'O methyl phosphorothioate chemistry.

P16 | NOVEL CAPSID SEQUENCES FROM ANCIENT AND CONTEMPORARY ADENO-ASSOCIATED VIRUSES IN AUSTRALIAN MARSUPIALS

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Presenting Author: Claus V. Hallwirth

The broad scope of adeno-associated virus (AAV)-based vectors as powerful tools for genetic manipulation is evidenced by their successful application in diverse clinical trials and pre-clinical settings. AAV capsid proteins are the primary determinants of vector tropism, immunogenicity and intracellular trafficking of the DNA cargo. Hence, novel AAV variants are being developed to impart properties desirable for therapeutic applications, such as specificity for selected target tissues and enhanced capacity to deliver DNA templates for genome editing. We hypothesised that AAV isolates from Australian marsupials, given their evolutionary isolation, could constitute a hitherto untapped resource for further advancement of the AAV vector system, by providing (i) novel capsid sequences that can be directly tested in transduction studies; and (ii) valuable insight into AAV capsid evolution via comparison with known AAV capsids. Our previous discovery of an AAV-derived germline endogenous viral element (EVE) in macropodoid marsupials and the successful reconstruction of its inferred ancestral (~30 million-year-old) AAV sequence prompted the search for additional marsupial AAV-EVEs. To this end, we generated whole-genome

sequencing data from the common wombat and brushtail possum, and secured access to several unpublished marsupial genome assemblies, and demonstrated that all harbour AAV-related EVEs. We also identified a novel contemporary marsupial AAV (mAAV) from faecal samples, providing evidence of ongoing AAV infection amongst Australian marsupials. The deduced capsid sequences from the ancient macropodoid AAV and mAAV are being functionally characterised in terms of protein coding capacity, capsid assembly and functional transduction of a selection of human and marsupial cell lines.

P17 | LONGITUDINAL TRACKING OF BONE MARROW-DERIVED CELL ENGRAFTMENT IN TSPO KNOCKOUT MICE WITH 18F-PBR111 MICROPET/CT: A NON-INVASIVE STUDY OF THE TURN-OVER OF HEMATOPOIETIC CELLS THROUGHOUT THE BODY

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Presenting Author: Meredith Harrison Brown

Tracking of stem cells and their progeny *in vivo* is important for the monitoring of engraftment success in both preclinical experiments and in clinical applications. Here we present a novel model of tracking bone marrow-derived cell engraftment *in vivo* using translocator protein (18kDa) knockout (TSPO$^{-/-}$) mice. TSPO$^{-/-}$ mice were irradiated and transplanted with nucleated bone marrow cells from age-matched, TSPO-expressing wild-type mice from a matched C57BL/6 background. At various stages of engraftment, the mice were anaesthetised, and injected with 0.2nM of ^{18}F-PBR111 over 1 minute via the tail vein. Dynamic scans were acquired for 50 minutes post-injection with an Inveon microPET/CT scanner, followed by whole-body CT scans for anatomical reference. PET images were reconstructed using a 4D deconvolution and denoising technique to correct for partial volume effect. Time-activity curves were extracted from two regions of interest drawn on co-registered PET/CT images corresponding to the spleen and thymus. ^{18}F-PBR111 microPET showed a clearly delineated, increased signal in the area of the spleen and thymus in TSPO$^{-/-}$ mice 1 month after bone marrow transplantation and remained so throughout the observation period of 12 months, indicating stable hematopoietic engraftment. These results were further corroborated by autoradiographic detection in tissue sections using ^{125}I-CLINDE, and immunohistochemical staining with a monoclonal TSPO antibody. This model, utilising TSPO$^{-/-}$ mice and a selectively binding TSPO radioligand (^{18}F-PBR111), demonstrates the successful, non-invasive tracking over time of transplanted hematopoietic cells throughout the body. This approach should thus also be feasible for the tracking of other TSPO-expressing stem cells and their progeny in models of disease or regenerative therapy.

P18 | COMPARISON OF THERAPEUTIC EFFICACY OF NEURAL PROGENITORS TO UMBILICAL CORD STEM CELLS IN A RODENT MODEL OF NEONATAL BRAIN DAMAGE

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Stem cell therapies have been postulated as a viable therapeutic tool in neuro-protection along with both neural repair and regeneration. While work with human umbilical cord blood cells (HuCBCs) can be described as safe, there is little evidence of substantial and consistent gain of function. Currently there is no preclinical work examining the differences in therapeutic efficacy between HuCBCs and pluripotent cells that have been differentiated to a neural lineage. It was the purpose of this study to elucidate these differences and determine whether any evidence exists for the therapeutic use of either or both cell sources following neonatal ischemic stroke. Motor and cognitive behaviour was assessed through rotarod, staircase, digigait and touchscreen methods. Holistically the data indicated that grafts of neural progenitors but not HuCBCs improved behavioural outcomes compared to ischemia alone. Systematic histological analysis was also performed. This analysis revealed a comparable level of atrophy among stroke induced groups at the target site, however a reduction in atrophy relative to lesion alone groups distal to the target site was observed in the group which received cortically differentiated pluripotent cells. This suggests a neuroprotective effect, one that was not present in the group which received cord blood cells. This analysis failed to find any surviving cord blood cells in the brain at either 7 days or 12 weeks of age, while cortically differentiated pluripotent grafts were present and displayed appropriate markers at both time points.

P19 | MODIFIED MUA TRANSPOSITION-BASED SEQUENCING USING THE ION-TORRENT PLATFORM TO IDENTIFY RETROVIRAL INTEGRATION SITES IN A CLINICAL GENE THERAPY TRIAL

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Presenting Author: Atul Kamboj

One major risk associated with integrating vectors in gene therapy is that of insertional mutagenesis, as was seen in children treated for X-linked severe combined immunodeficiency when vector integration within proto-oncogenes, most notably LMO2, resulted in development of T cell leukaemias. Factors that contribute to this risk include the underlying integration behaviour of the vector type, target cell phenotype, transduction conditions, vector cassette design and transgene cargo. Analysis of vector integration sites (ISs) is therefore critically important in both monitoring the safety of patients undergoing gene therapy and to inform choice of vectors for clinical use. Next generation sequencing (NGS) is a valuable tool for mapping vector ISs to address these aims. We have developed a Mu transposition-based library preparation method suitable for sequencing using the IonTorrent platform. Using this method, coupled to a UNIX-based analysis pipeline to map ISs, we have generated IS datasets for haematopoietic stem cells (HSC) transduced as part of our Phase I Cancer Gene Therapy trial. Comparisons of ISs detected in samples taken from gene-modified cell products that patients received, identify 70 common genes that were targeted for integration with our clinical vector across four patients. Sixty-five of these were found to be common with another independent study in which HSCs were transduced with the same vector under differing transduction conditions. NGS using MuA transposition-based amplification provided an efficient and reproducible approach to recover the ISs, and provides a robust method by which vector safety can be assessed and estimated for future trials.

P20 | DELIVERY OF MUSCLE PROGENITOR CELLS BY TROJAN HORSE MICROTISSUE CONSTRUCTS: TOWARDS AUTOLOGOUS REGENERATIVE CELL THERAPIES FOR MUSCLE DISEASE

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Presenting Author: Quigley A

Muscle degeneration due to denervation or disease processes such as Muscular Dystrophy result in progressive loss of muscle tissue. Advancements in gene editing (GE) and induced pluripotent stem cell (iPSC) technologies over the past 17 years have made possible the generation of autologous myogenic precursors for regenerative cell implants into diseased muscle. The majority of regenerative strategies for rebuilding muscle use cell replacement therapies where progenitor or stem cells are injected directly into the muscle or the blood stream. These approaches are limited as a large percentage (>80%) of the injected cells die before they can establish functional muscle fibres in the recipient and/or too few donor cells participate in muscle regeneration.

In our recent work, we have used additive bio-fabrication technologies to develop cell laden hydrogel based fibres and scaffolds that facilitate cell survival and proliferation. *In vitro*, these scaffolds support cell growth and division of encapsulated progenitor cells for at least 3 weeks with high rates of cell proliferation. We have used this delivery method, in combination with manipulation of the regenerative environment of the muscle, to deliver non-dystrophic donor myogenic progenitors to skeletal muscle of dystrophic mice. Using this approach, we found that large numbers of donor-derived dystrophin-positive muscle fibres persisted for at least 12 weeks and have achieved up to approximately 50% remodelling of recipient muscles using a relatively low number of donor cells.

Manipulation of the regenerative environment of the muscle and transplantation of donor cells within a protective scaffold facilitates muscle progenitor expansion and engraftment and presents an effective approach for regenerative (functional) cell delivery to regenerating muscle. This approach presents an alternative method of functional precursor cell delivery to muscle and in conjunction with effective iPSC and GE technologies, provides a firm basis for effective autologous ***regenerative*** cell therapy for muscle disease.

P21 | HUMAN AMNION EPITHELIAL CELLS PROTECT IMMATURE OLIGODENDROCYTES IN A MODEL OF PERINATAL BRAIN INJURY

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Presenting Author: Bryan Leaw

Cerebral palsy is the leading cause of physical disability, affecting 1 in 500 births. Delayed white matter maturation is the precedent for motor and learning impairment later in life. As this is caused by inflammation and subsequent cell death, targeting these may have therapeutic benefit. Human amnion epithelial cells (hAECs) are clonogenic and we showed previously that hAECs reduce apoptosis and glia activation. Here, we investigate the effects of hAECs on oligodendrocytes and microglia surveillance, which is known to affect myelination.

Methods: On embryonic day 16 we administered lipopolysaccharide (LPS), or saline (control), intra-amniotically to C57Bl/6J mouse pups. After birth, LPS pups were placed in hyperoxia (65% oxygen) and control pups in normoxia for 14 days. Pups were given either hAECs or saline intra-venously on P4. For slice culture experiments, brains were isolated from adult CX3CR1 mice. Brain slices were cultured at 37°C in 5% CO2 for 12 hours. Cell tracking was performed using IMARIS.

Results: At P14, brains from LPS and hyperoxia pups had reduced expression of the immature oligodendrocyte marker, CNPase. There

was no change in mature myelin (MBP). In hAEC-treated animals, there was increased CNPase expression in the corpus callosum and external capsule, suggesting that hAECs might influence oligodendrocyte maturation, with no effect on MBP. We next assessed microglia behavior after hAEC conditioned medium (hAEC-CM) treatment. hAEC-CM resulted in increased expression of CX3CR1, increased microglia recruitment as well as total displacement and speed. This data show that hAECs are able to directly influence microglia surveillance, critical to resolution of pathology, and may influence myelination.

P22 | SMALL CHEMICAL MODULATION OF INDUCED ALTERNATIVE SPLICING

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Presenting Author: Dunhui Li

Splicing switching antisense oligonucleotides (AOs) are showing great therapeutic potential to treat inherited diseases, with Exondys 51 (a phosphorodiamidate morpholino oligomer) recently being granted accelerated approval by the US FDA for the treatment of Duchenne muscular dystrophy (DMD). The longest running clinical trial is now in its 5th year, and although there is an unequivocal increase in dystrophin after treatment, the amounts are modest and there is room for improvement. We are exploring the possibility of a combinatory therapy using the AOs and FDA approved drugs known to influence splicing. The SR protein family of splicing factors has important roles in both constitutive and alternative splicing regulations, with post-translational modifications (phosphorylation, acetylation, methylation) of SR proteins, influencing RNA splicing. Riluzole, an FDA approved neuro-protective drug for Amyotrophic lateral sclerosis (ALS), was reported to inhibit CDC2-like kinases (CLK family) and disrupt the phosphorylation status of specific SR proteins that promotes the recognition of nearby 5' and 3' splice site by U1 and U2 snRNP. Our preliminary studies demonstrate that pretreatment of primary human myotubes with Riluzole can enhance 2OMe induced exon 51 skipping in normal human primary myogenic cells in a dosage dependent manner. Further studies are under way to further refine timing and Riluzone:AO dosages and explore the underlying mechanism, paving the way for novel combinatory therapies to safely enhance AO induced exon skipping as a treatment for DMD.

P23 | THE VECTOR AND GENOME ENGINEERING FACILITY (VGEF): CUSTOMISED SERVICE FOR YOUR RESEARCH NEEDS

Predrag Kalajdzic[1]; Kimberley Dilworth[1]; Grober Baltazar Torres[1]; Ian E. Alexander[2]; Patrick P.L. Tam[3]; Leszek Lisowski[1,4,5]

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Presenting Authors: Predrag Kalajdzic, Kimberley Dilworth, Grober Baltazar Torres

The mission of the Vector and Genome Engineering Facility (VGEF), a recently established research core of the Children's Medical Research Institute, is to support basic and translational research in vector-based gene therapy and genome engineering. In addition to being a service facility that provides investigators the access to the latest vector and genome engineering technologies, VGEF is also active in developing new, and improving existing, technologies that will benefit its clientele and the wider scientific community.

The VGEF facility offers customised preparations of lentiviral (LV), adenoviral (Ad), and adeno-associated viral (AAV) vectors, at purities and production scales tailored to the needs of individual projects to facilitate both *in vitro* and *in vivo* experimentation, as well as ready-to-use AAV and LV stocks.

In addition to vector-based tools, VGEF offers genome-editing services in mouse and human cell lines, mouse ES cells and human iPS cells. VGEF closely follows and utilises the latest advances in genome editing technologies. Currently VGEF uses a range of CRISPR/Cas9-based systems, such as custom Cas9 nucleases for precise genome modifications, and endonuclease-free AAV-mediated editing.

In alignment with our goal to promote vector-based and genome engineering technologies, we offer consultation in study design and technical support in the selection of appropriate tools for specific experimental needs, as well as hands-on training opportunities. Beyond our academic and commercial services, we also engage in collaborative studies that are aligned with our research focus in AAV biology, vector development and genome engineering.

P24 | OPTIMISATION OF KIDNEY ORGANOIDS AS A REPRODUCIBLE AND DEVELOPMENTALLY ACCURATE MODEL OF HUMAN KIDNEY DEVELOPMENT AND DISEASE

Melissa H. Little[1,2]; Pei X. Er[1]; Belinda Phipson[1]; Jessica Vanslambrouck[1]; Sara Howden[1]; Lorna Hale[1]; Minoru Takasato[1]; A. Oshlack[1]

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Presenting Author: Melissa H Little

The generation of complex tissue organoids via directed differentiation of human pluripotent stem cells brings the prospect of personalised

drug testing, disease modelling and regenerative medicine. We have developed a protocol for the generation of kidney organoids comprised of nephrons, collecting duct, vasculature and surrounding interstitium (Takasato et al, Nature, 2015) from human pluripotent stem cells. This protocol relies upon the stepwise recapitulation of morphogenetic events previously characterised during normal kidney development in the mouse. The utility of the protocol for applications such as the modelling of human kidney disease will rely implicitly on the accuracy of this differentiation program, the reproducibility of the directed differentiation, the transferability between iPSC lines and the functional authenticity of the human cell types generated. Using RNA-seq based transcriptional analyses across the differentiation timecourse from pluripotent iPSC to kidney organoid, we have examined transcriptional variation between individual organoids, distinct differentiation experiments and between cell lines. Gene ontology interrogation of unsupervised clusters of synexpression, as well as pairwise temporal changes in gene expression, show a clear transition through appropriate intermediate developmental patterning events, including primitive streak, intermediate mesoderm, metanephric commitment and nephrogenesis. This confirms the high correlation between human and mouse metanephrogenesis, and provides a framework for quality control. A transcriptional correlation of >95% was observed between organoids and >90% between distinct differentiation experiments. Distinct iPSC clones also conformed to a matching transcriptional program. An examination of the most variable genes shows that these represent genes characteristic of nephron patterning, suggesting differential organoid maturation as the major source of both inter-experimental and intra-clonal variation. This data provides a framework for removing sources of unwanted experimental variation in the analysis of expression data, thereby increasing the utility of this approach for personalised medicine and functional genomics.

P25 | GENOME-WIDE MAPPING OF REGULATORY TRANSCRIPTIONAL NETWORKS IN HUMAN ENDOMETRIAL MESENCHYMAL STEM/STROMAL CELLS BY ATAC-SEQ AND RNA-SEQ

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Presenting Author: Raffaella Lucciola

The extraordinary regenerative ability of the endometrium to adapt to changes, physiological or otherwise, depends on endometrial mesenchymal stem cells (eMSCs) with extensive self-renewal and differentiation capacity. Deficiency in eMSCs has been linked to reproductive failure. However, the transcription factors (TFs) and signalling pathways that maintain stemness of eMSCs are poorly understood. We previously demonstrated increased eMSC proliferation upon inhibition of transforming growth factor-β receptor (TGF-β R) signalling. In this work, primary eMSCs were treated with and without TGF-β R inhibitor (A83-01). Increased population doublings of cultures treated with A83-01 confirmed that TGF-β signalling negatively regulates eMSC expansion. Flow cytometry for 3 eMSC markers showed that A83-01 increases the % of SUSD2- and CD140b-positive cells (63% and 99%, respectively) in prolonged cultures, which was paralleled by higher clonal efficiency. Combined RNA- and ATAC- (Assay for Transposase-Accessible Chromatin) sequencing was used to identify TFs and target genes regulated by A83-01. A total of 1,463 differentially expressed genes were identified following A83-01 treatment of primary eMSCs for 5 weeks. Gene ontology revealed that up- and down- regulated genes are enriched, amongst others, in 'development' and 'structural' categories, respectively. Preliminary ATAC-seq analysis showed that A83-01 triggers opening and closure of 3,555 and 2,412 chromatin loci, respectively; with significant enrichment for binding sites for various TFs, including RAR/RXR and CEBPB/D, matching the induction of RARB, RORA and CEBPB/D. Taken together, our study has provided new insights into the gene networks and genome-wide chromatin landscape associated with stemness of cultured eMSCs.

P26 | DEVELOPMENT OF A NON-INVASIVE LIQUID BIOPSY FOR PROSTATE CANCER: IDENTIFICATION OF EXOSOME MICRORNA (EXOMIR) SIGNATURES IN PATIENT BODY FLUIDS FOR CANCER DIAGNOSIS AND PROGNOSIS

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Presenting Author: Rosetta Martiniello-Wilks

Prostate cancer (PCa) diagnosis and prognosis currently relies on a Prostate Specific Antigen blood test which is unreliable in some men, in addition to an invasive prostate biopsy leading to poor prostate health check compliance. To overcome these hurdles, we have developed a unique workflow for profiling the microRNA content of cell line supernatant and human urine exosomes (exomiR) with the aim of discovering advanced PCa biomarkers for the development of a non-invasive liquid biopsy. Exosomes were isolated using ultracentrifugation from the supernatant of transformed prostate epithelium and a number of androgen sensitive (LNCaP) or independent (PC3, DU145, VCaP) PCa cell lines. Urine exosomes were isolated using ultrafiltration and ultracentrifugation from samples collected with consent from healthy, age matched donors and PCa patients before and after radical prostatectomy. NanoSight analysis showed an enrichment of particles

of expected exosome size with minimal contamination by other vesicle types. Europium exosome capture assays confirmed enrichment with abundant exosome marker CD9 and TSG101 expression, while free microRNA bound AGO2 contamination could not be detected. Exosome RNA quality was confirmed using Bioanalyzer Pico Chips prior to qPCR profiling using Affimetrix microRNA microarrays or Applied Biosystems TaqMan based microRNA OpenArrays. Microarray analysis of urine exosomes (OpenArray) revealed 24 potential PCa exomiR where the cell line analysis revealed 10 potential biomarkers (Affimetrix). Interestingly no overlap in these exomiR signatures was observed highlighting the importance of using patient samples for clinical PCa biomarker discovery. Thus urine exomiR show promise as a source of diagnostic/prognostic biomarkers to support the development of a novel non-invasive liquid biopsy which will fill a significant gap in the clinical care of PCa patients.

P27 | RESCUING CFTR FUNCTION CAUSED BY MUTATION SPECIFIC POLYMORPHISMS IN CYSTIC FIBROSIS PATIENTS

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Presenting Author: Kelly M Martinovich

Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations result in cystic fibrosis (CF) disease and a broad spectrum of clinical phenotypes are observed in CF patients. One mutation, Arg117His affects the conductivity of the CFTR channel and can result in a mild or severe phenotype, influenced by co-location of the mutation and an intron 9 polymorphism: a poly T tract varying from 5 to 9 nucleotid. The shorter '5T' allele weakens the intron 9 acceptor site and promotes exclusion of exon 10 from the mature *CFTR* transcript, resulting in a non-functional CFTR channel, and a more severe disease phenotyp. Manipulation of *CFTR* pre-RNA splicing using antisense oligonucleotides (AOs) is a potential therapy for those CF patients with this particular mutation. This study explores antisense therapy to correct abnormal splicing of *CFTR* RNA and improve CFTR function. AOs targeting *CFTR* intron 9, around the specific 5T polymorphism, were designed and transfected into monolayer primary airway epithelial cells from a CF patient harbouring this disease-associated polymorphism. AOs that decrease the levels of *CFTR* transcript missing exon 10 will then be assessed for CFTR function using an Ussing Chamber and CF patient primary epithelial cells grown at the Air-liquid interface.

We propose that corrected splicing of the *CFTR* 5T allele will improve function in CF patients carrying selected mutations, either alone or in combination with current therapeutics.

P28 | TARGETED THERAPY FOR OSTEOSARCOMA IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS

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Presenting Author: Shiloh Middlemiss

Osteosarcoma is a cancer paediatric patient's which is difficult to treat, especially when refractory and metastatic disease is present at diagnosis. With 5-year event free survival remaining relatively low at 50-60%, there is an urgent need for targeted therapies to improve survival. Our focus is the development of immune therapies to target osteosarcoma, using patient T cells bearing tumour specific Chimeric Antigen Receptors (CAR-T cells) against Erythropoietin-producing Hepatocellular receptor tyrosine kinase class A2 (EphA2). This protein is expressed at varying levels on osteosarcoma cells and in other solid tumours, and is implicated in both oncogenesis and tumour angiogenesis, making it a promising target for CAR-T cells.

Our lab has developed a lentiviral construct, and methods for culturing and gene modifying T cells, to successfully generate functioning EphA2 CAR T cells that can target osteosarcoma cells. Cytotoxic responses by EphA2 CAR T cells against EphA2 positive osteosarcoma cells lines result in cell destruction *in vitro*. Furthermore, Enzyme Linked ImmunoSorbent Assays (ELISAs) confirmed EphA2 CAR –T cell activity and function with the release of the cytokines Interleukin-2 (IL-2) and Interferon-γ (IFN-γ) by EphA2 CAR -T cells following co-incubation and engagement of the EphA2 protein expressed on the osteosarcoma cells. Current work to test EphA2 CAR- T cell function and efficacy in osteosarcoma cells *in vivo*, in ectopic and orthotopic xenograft mouse models of osteosarcoma, are underway. In addition, evaluation of EphA2 expression on other paediatric solid tumours may broaden the target range of EphA2 CAR T cell in a paediatric setting.

P29 | DEVELOPING CELL AND GENE THERAPY TO TREAT FRIEDREICH ATAXIA

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Presenting Author: Sze Hwee Ong

Friedreich ataxia (FRDA) is a debilitating neurodegenerative disorder that typically begins ≈10 years of age and is characterised by progressive gait ataxia. Affected individuals become wheelchair-dependent within 15 years of disease onset and have a markedly shorter lifespan of average 37 years, with cardiomyopathy being the main cause of death. With no current treatment which can cure or slow the neurodegeneration inherent to FRDA, treatments that slow FRDA disease progression are urgently needed. FRDA is most often caused by homozygous GAA repeat expansions in intron 1 of *FXN*. As the GAA expansion decreases the encoded frataxin protein, but does not alter the protein sequence or structure, increasing frataxin will likely be beneficial. Using cell and animal models, this project aims to increase frataxin to alleviate the FRDA phenotype via cell and gene therapy. Lethally irradiated FRDA mice have been transplanted with either GFP-positive wild-type bone marrow (BM; corrected) or FRDA BM (non-corrected). Reconstitution of the haematopoietic system demonstrated successful engraftment in corrected and non-corrected mice. In corrected recipients, GFP-positive cells were observed in the spinal cord and dorsal root ganglia (DRG), key tissues of neuropathology. DRG neuronal marking, particularly proprioceptive neurons increased while frataxin levels remained unchanged. Corrected mice also exhibited significant improvement in locomotor activity post-bone marrow transplant (BMT). Toward developing autologous gene therapy via BMT, we constructed a lentiviral vector that expresses frataxin. These data together illustrate the corrective potential of BMT to treat FRDA and provide an avenue for delivering therapeutic viral vectors for autologous gene therapy.

P30 | LARGE SCALE PRODUCTION OF RECOMBINANT AAV USING THE BACULOVIRAL EXPRESSION VECTOR SYSTEM

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Presenting Author: Renuka Rao

Recombinant AAV (rAAV) is one of the most promising viral vectors for somatic gene therapy due to its lack of pathogenicity and long term transgene expression. Triple plasmid transfection method in HEK293 cells is the most commonly used method for production of rAAV. However, scalable production using this method is a major challenge in the use of rAAV for clinical trials. In this study, we used the insect cell (Sf9)/baculovirus expression system as described by Kotin et al (Mol Ther. 2009 Nov;17(11):1888-96). Four recombinant baculoviruses, namely a Bac AAV transgene containing eGFP driven by the CBA promoter and a WPRE sequence at the 3' end flanked by the ITRs and three Rep/Cap Bacs with Rep2 and codon modified capsids 6,8 and 9 were generated. rAAV-GFP for each serotype was produced by double infection of Sf9 insect cells with the Bac transgene and the appropriate Rep/Cap Bac. 96h post infection, rAAV was purified from the cell pellet and supernatant by either 2%PEG or ammonium sulphate precipitation, followed by cesium chloride density gradient centrifugation. Quantitative PCR showed that insect cells produced equivalent amounts of rAAV genomes for all the 3 capsids as that produced by HEK293 triple plasmid transfection system. Transduction efficiency to determine functional rAAV was carried out in highly permissive 2v6.11 cells. The data suggests expression of GFP to be lower in Baculo derived rAAV as compared to HEK derived rAAV. Further experiments are required to elucidate whether this translates to a difference in *in vivo* transduction efficiency.

P31 | OSTEOIMMUNOMODULATORY PROPERTIES OF OSTEOGENICALLY DIFFERENTIATED MSCS ON THE PHENOTYPIC SWITCHING OF MACROPHAGES

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Presenting Author: Rong, H

Background: The bone remodeling process requires a precise regulatory network among bone marrow mesenchymal stem cells (MSCs), immune cells and bone cells. Recent studies have shown that macrophages can cause the activity changes of osteoclasts and osteoblasts. However, it remains unknown whether macrophages are affected by MSCs during osteogenesis, leaving a certain gap unfilled in osteoimmunology.
Methodology: A mouse skull defect model was used to unveil macrophage recruitment following transplantation of MSCs and osteogenically differentiated MSCs (O-MSCs). The transwell and xCelligence system were applied to study the effect of O-MSCs on cell motility. Cytokines and cell surface markers induced with O-MSC conditioned medium were examined by qRT-PCR and FACS. Identification of the signaling pathway was conducted using lentivirus vector targeting VEGF.
Results: Our results demonstrate that O-MSCs regulated the recruitment of macrophages through the secretion of VEGF both *in vivo* and *in vitro*. The secretion of pro-inflammatory cytokines in macrophages was significantly enhanced under O-MSCs stimulation. VEGF know-down led to the decrease of RANKL expression and further affected the phototype of macrophages. Neutralisation of VEGF in skull defects led to a significant decrease of the number of M1 macrophages and new bone formation.
Conclusions: Our study provides a new perspective on the immunoregulatory function of O-MSCs and the potential cytokines involved in the regulation such as VEGF and RANKL. Targeting VEGF and

RANKL may be a promising strategy to manage bone defects and lead to improved therapeutic outcomes.

P32 | BIMODAL BEHAVIOUR OF INTERFOLLICULAR EPIDERMAL PROGENITORS REGULATED BY HAIR FOLLICLE POSITION AND CYCLING

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Presenting Author: Roy Edwige

Interfollicular epidermal (IFE) homeostasis is a major physiological process that allows the maintenance of the skin barrier function. Despite progress in our understanding of stem cell populations in different hair follicle compartments, cellular mechanisms of IFE maintenance, in particular, whether a hierarchy of progenitors exists within this compartment, have remained controversial. We here used multi-colour lineage tracing with Brainbow transgenic labels activated in the epidermis to track individual keratinocyte clones. Two modes of clonal progression could be observed. Clones attached to hair follicles showed rapid increase in size during the growth phase of the hair cycle. On the other hand clones distant from hair follicles were slow-cycling, but could be mobilized by a proliferative stimulus. Reinforced by mathematical modelling, these data support a model where progenitor cycling characteristics are differentially regulated in areas surrounding or away from growing hair follicles. Thus, while IFE progenitors follow a non-hierarchical mode of development, spatiotemporal control by their environment can change their potentialities, with far reaching implications in our understanding of epidermal homeostasis, wound repair and cancer development.

P33 | IMMUNOMODULATORY AND ANTI-INFLAMMATORY CAPABILITIES OF CANINE MESENCHYMAL STEM CELLS AND THEIR CLINICAL POTENTIAL

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Presenting Author: A. Shahsavari

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have acquired an important role in cell-based therapeutics in both humans and dogs While human MSCs are immunomodulatory and have been shown to be potent regulators of inflammation via active suppression of both the innate and adaptive immune systems, very little is known about the immunomodulatory and anti-inflammatory abilities of canine MSCs. Similar findings in canine MSCs might facilitate the use of these cells as a treatment for immune-mediated and inflammatory diseases such as osteoarthritis, inflammatory bowel disease and atopy, common diseases in dogs and humans. MSCs are typically collected from adipose tissue (AT-MSCs) and bone marrow (BM-MSCs). However, in an effort to avoid the issues of variable quality and quantity of harvested canine MSCs, we have generated MSCs from canine iPSCs (iPSC-MSCs). In this study, we examined the expression of immunomodulatory and anti-inflammatory cytokines in iPSC-MSCs as compared to harvested AT- and BM-MSCs. The expression by iPSC-MSCs of key immunomodulatory and anti-inflammatory cytokines such as indoleamine 2, 3 dioxygenase (IDO), transforming growth factor β (TGFβ), prostaglandin receptor 2 α (PTGER2α), cyclooxygenase 2 (COX2) and hepatocyte growth factor (HGF) is comparable to, or greater than, that observed in harvested AT- and BM-MSCs. The effects of iPSC-, AT- and BM-MSCs on mixed canine lymphocyte cultures were also examined. Based on our data, canine iPSC-MSCs are an ideal candidate for further studies towards developing cell-based therapies for regulating immune-mediated and inflammatory diseases in the dog.

P34 | DEVELOPMENT AND EVALUATION OF LENTIVIRAL VECTORS TARGETING THE B CELL COMPARTMENT FOR THE TREATMENT OF X-LINKED AGAMMAGLOBULINAEMIA

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X-linked agammaglobulinaemia (XLA) is a primary immune deficiency characterised by the absence of mature B cells and results from mutations in the Bruton tyrosine kinase (*BTK*) gene. Components of the B-cell receptor (BCR) signaling pathway, including *BTK* functionality, can be assessed by changes in intracellular calcium concentration (calcium flux). Calcium (Ca^{2+}) signaling in B-cell activation regulates proliferation, differentiation, apoptosis and multiple Ca^{2+}-sensitive transcription regulators. Following stimulation with IgM, cells expressing BTK also exhibit a positive Ca^{2+} flux.

To determine the feasibility of *ex vivo* gene therapy for XLA, we have developed a third-generation lentiviral vector expressing human codon-optimised BTK and transduced XLA patient $CD34^{+}$ progenitor cells. These cells were engrafted into NOD/SCID/gamma (NSG) mice

and the level of B cell reconstitution was determined. A positive Ca^{2+} flux was found in mice transplanted with wild-type human $CD34^{+}$ cells and in at least one of three mice transplanted with XLA patient cells following transduction, albeit at a low level. This result indicated that, in mice transplanted with gene-modified XLA patient progenitors, the BCR signaling pathway had been restored in some cells, suggesting that the vector-encoded BTK was functional. To optimise B-cell reconstitution in engrafted NSG mice, a panel of lentiviral vectors was constructed and evaluated for promoter strength and B cell lineage-restricted expression. High Ca^{2+} flux was observed in a Btk-deficient cell line (DT40) when BTK expression was driven by the BTK promoter, and preferential B cell expression was observed in mice when reporter gene expression was driven by the CD45 promoter.

P35 | NITRIC OXIDE DIRECTED REPROGRAMMING OF RAT BONE MARROW DERIVED MESENCHYMAL STEM CELLS INTO ENDOTHELIAL-LIKE CELLS VIA ACTIVATION OF WNT/B-CATENIN SIGNALING

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Presenting Author: Padraig Strappe

Nitric oxide plays an important role in stem cell differentiation, and is produced in endothelium by endothelial nitric oxide synthase (eNOS) located within membrane caveolae bound with caveolin-1 ($CAV\text{-}1^{WT}$) which inhibits NO production. Rat bone marrow mesenchymal stem cells (rBMSCs) were modified with integrating and non-integrating lentiviral vectors to co-express eNOS and a non-inhibitory mutant caveolin-1 ($CAV\text{-}1^{F92A}$) to enhance NO generation and investigate endothelial reprogramming. A lenti-eNOS-GFP vector allowed FACS selection of eNOS positive cells which were further transduced with $CAV\text{-}1^{F92A}$ resulting in $rBMSC^{eNOS+CAV\text{-}1F92A}$ cells which showed increased NO production, enhanced *in vitro* tubule formation, CD31, V-cadherin gene expression and activity of an endothelial specific FLT1-eGFP lentiviral-reporter. Arterial specific Notch1, Hey 1 and Dll4 gene expression was increased in $rBMSC^{eNOS+CAV\text{-}1F92A}$ whereas venous COUP-TFII and lymphatic Prox1 expression was reduced. Canonical Wnt3A gene expression was increased in $rBMSC^{eNOS+CAV\text{-}1F92A}$ and treatment with the eNOS inhibitor L-NAME, resulted in reduced CD31 and Wnt3A expression, and the Wnt inhibitor Dkk-1 also decreased CD31 expression. β-catenin protein expression was increased in $rBMSC^{eNOS+CAV\text{-}1F92A}$ confirming the relationship between NO and Wnt/β-catenin signaling. Furthermore $rBMSC^{eNOS+CAV\text{-}1F92A}$ cells showed decreased histone deacytaylase SIRT6 and DNA methyltransferase DNMT1 expression which was reversed with L-NAME treatment highlighting that NO may drive epigenetic modification during reprogramming. Finally, subcutaneous implantation of the $rBMSC^{eNOS+CAV\text{-}1F92A}$ cells seeded in polyurethane scaffolds in rats, resulted in formation of larger blood vessels compared to controls. In summary, nitric oxide may activate Wnt/-β-catenin signaling to promote endothelial reprogramming of BMSCs and may provide a novel platform for vascular repair.

P36 | DEFINING AN ALLOGENEIC CAR-T APPROACH BY SHRNA-MEDIATED KNOCKDOWN OF THE T-CELL RECEPTOR

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Background: An allogeneic CAR T-Cell therapy can streamline manufacturing, provide greater accessibility to patients and enhance safety by reducing graft-versus-host-disease. The T-Cell Receptor (TCR) is comprised of multiple subunits and functions to activate T-cells by a signal transduction cascade initiated upon antigen binding. Eliminating the endogenous TCR on modified CAR-T-Cells may eliminate the ability to recognize major and minor histocompatibility antigens in the recipient. This study assessed if simultaneous expression of multiple short hairpin RNAs (shRNA) that knockdown levels of individual TCR subunits results in loss of TCR expression and TCR-mediated T-Cell activation.
Methods: Recombinant DNA producing shRNA against the TCR was transfected into T-cells. Cell surface TCR was analyzed by FACS. Following CD3 activation, T-Cell activation was quantified by assessing levels of IL-2.
Results: Expressed individually, each shRNA inhibited it's cognate TCR subunit up to 93%. Simultaneous expression of shRNAs against different subunits resulted in near complete depletion of the TCR from the cell surface as measured by FACS. IL-2 secretion was inhibited to undetectable levels by ELISA by the multi-shRNA treatment and >98% by qPCR.
Conclusions: Though knockdown of any single component of TCR never exceeded 93%, simultaneous knockdown of several TCR subunits abrogated surface TCR and downstream activation suggesting that disruption of stoichiometric expression the subunits prevented TCR formation. The small size of shRNA expression cassette (<2 Kb) permits co-expression from the same lentiviral vector as the CAR. Altogether, these data point to a strategy towards generating a single vector approach for producing allogeneic T-Cells for immunotherapies.

P37 | SIGNIFICANT ENHANCEMENT OF HEPATITIS B VIRUS (HBV) SUPPRESSION WITH STANDARD OF CARE DRUGS FOLLOWING CO-ADMINISTRATION OF A DNA-DIRECTED RNA INTERFERENCE AGENT IN A CHIMERIC MOUSE MODEL

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Presenting Author: David Suhy

Background: BB-103 is an AAV8 vector expressing steady state levels of three anti-HBV shRNAs that target well-conserved sequences on HBV RNA. We examined if combinations of BB-103 and current standard of care drugs could significantly impact HBV parameters.

Methods: PXB mice have chimeric livers comprised of >70% human hepatocytes permitting sustained HBV infection. BB-103 was administered once, at the beginning of treatment, and anti-HBV activity was monitored over the course of 13 weeks by following serum HBV DNA, HBsAg and HBeAg weekly. In combination studies, a single dose of BB-103 was administered with daily entecavir (ETV) or twice weekly treatments of pegylated interferon (PegIFN).

Results: As a monotherapy, BB-103 caused a 2.17 log drop in serum HBV DNA. Modest rebounds of HBV DNA levels were noted after 56 days. ETV monotherapy resulted in a 2.63 log drop in serum HBV DNA levels. Remarkably, a single dose of BB-103 in combination with daily ETV dropped the serum HBV DNA levels well below 3.72 logs, the LLOQ. More importantly, BB-103+ETV dropped HBsAg levels, a known contributor to immunosuppression and HBV chronicity, by 2.14 log versus a 0.46 log drop from ETV monotherapy. Co-treatment of mice with PegIFN and the ddRNAi compounds led to less substantial drops, perhaps reflecting on the putative mechanism of action of interferon.

Conclusions: Using a chimeric mouse model, we demonstrate that the addition of a single dose of BB-103 significantly enhances the anti-HBV efficacy of standard of care drugs and offers a promising path forward for disease treatment.

P38 | TOWARDS DEVELOPMENT OF A 'SILENCE AND REPLACE' BASED APPROACH FOR THE TREATMENT OF OCULOPHARYNGEAL MUSCULAR DYSTROPHY

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, late-onset muscle disorder characterized by ptosis, swallowing difficulties and proximal limb weakness. OPMD is caused by a trinucleotide repeat expansion that results in an N-terminal expanded polyalanine tract in the polyA binding protein nuclear-1 (PABPN1) gene, which encodes an ubiquitously expressed polyadenylation factor involved in many biological processes. No cure is currently available for OPMD. The cricopharyngeal myotomy is the only treatment available to improve swallowing in these patients, but the pharyngeal musculature still undergoes progressive degradation leading to death following swallowing impairment, pulmonary infections and choking. The A17 mouse model, expressing a modified bovine PABPN1 with an expanded polyalanine tract, recapitulates most of the features of human OPMD patients including a progressive atrophy and muscle weakness associated with nuclear aggregates of insoluble PABPN1. We describe here a gene therapy based approach to treat OPMD with an AAV-based strategy that relies upon a combination of silencing the endogenous PABPN1 with the expanded polyalanine tract via an RNAi based approach with the ability to express a codon optimized version of the wildtype protein. Using local intramuscular injections in the A17 model, we demonstrate that this 'silence and replace' methodology reduces nuclear aggregates, limits fibrosis as well as provides restoration of muscle strength to wildtype levels in this disease model. Collectively, these data provide compelling motivation for the continued development of a gene therapy approach to treat OPMD.

P39 | PRECLINICAL EVALUATION OF A BMSC DELIVERED GENE-DIRECTED ENZYME PRO-DRUG THERAPY (GDEPT) LOCAL BYSTANDER EFFECT IN IMMUNE INTACT MOUSE MODELS OF PRIMARY AND METASTATIC PROSTATE CANCER

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One in nine Australian men will be diagnosed with prostate cancer (PCa) in their lifetime and most deaths result from the spread of PCa to other organs (metastasis). Bone marrow mesenchymal stem cells (BMSC) show promise as GDEPT transfer vehicles due to their ability to preferentially migrate to and localise with cancer anywhere in the body while evading rejection by the immune system, a major hurdle of most viral gene delivery systems currently under investigation. We have modified bone marrow derived mesenchymal stem cells (BMSC) with a yeast prodrug converting enzyme gene *Fcy:Fur* which converts non-toxic prodrug 5-fluorocytosine (5FC) into a cell membrane permeable toxic metabolite 5-fluorouracil (5FU) that causes comprehensive cancer cell bystander killing, otherwise known as the local bystander effect.

Briefly BMSC were nucleofected with cell tracking reporter gene firefly luciferase (*fl*) and *Fcy:Fur* which encodes cytosine deaminase and uracil phosphoribosyltransferase (BMSC-Fcy:Fur-fl). RM1 prostate cancer cells (RM1) were modified with another cell tracking reporter gene renilla luciferase (*rl;* RM1-rl). This is the first study reporting

BMSC-Fcy:Fur-fl local bystander efficacy *in vivo* utilising a syngeneic and immune intact albino C57BL/6 mouse model of primary subcutaneous (sc) and pseudo-metastatic PCa.

Mice were implanted sc with either RM1-rl cells alone or a mixture of RM1-rl/ BMSC-Fcy:Fur-fl cells and tumours were allowed to establish. Mice were then treated systemically with 5FC or saline for 10 days. Sequential IVIS Lumina II bioluminescence full body imaging (BLI; Caliper/ Thermo Scientific) was used to quantify and track the local bystander effect of BMSC-Fcy:Fur-fl ± 5FC on RM1-rl tumour growth. Light reactions produced by RM1-rl substrate coelentrazine supported by tumour volume measurements showed a significant reduction in PCa tumour growth (98%) and improved survival in 75% of mice in the presence of BMSC-Fcy:Fur-fl/5FC when compared to the BMSC-Fcy:Fur-fl/saline control group.

To develop RM1 lung pseudo-metastases, mice were implanted with RM1-rl cells via the tail vein (day 0). Following lung lesion establishment, these mice then received a total therapeutic dose of 1-2x10^7 BMSC-Fcy:Fur-fl/kg/mouse (day 4-6). Mice were then treated with 5FC or saline for 6 days (day 7-12). Lung colony counts were performed to determine impact of the BMSC-Fcy:Fur-fl/5FC local bystander effect on lung pseudo-metastases. Comparison with the control group not receiving 5FC showed BMSC delivered GDEPT significantly reduced (67%) lung pseudo-metastases development in immune intact WB6 mice on day 12. BLI imaging showed tail vein infused BMSC-Fcy:Fur-fl cells migrated to lung pseudo-metastases and persisted in these lungs when compared to normal lungs of control mice. These results were confirmed by genomic DNA PCR of the *Fcy:Fur* gene in the major organs harvested from these mice. Thus, BMSC-Fcy:Fur-fl/5FC provided comprehensive local and metastatic PCa tumour killing *in vivo* and shows promise for the future development of a novel biotherapeutic for clinical care of patients with metastatic PCa.

P40 | PARTHENOGENETIC HUMAN EMBRYONIC STEM CELL LINES IN AUSTRALIA

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Parthenogenetic embryonic stem cell lines (pESC) can be made from mature human oocytes obtained from ovaries primed by hormones during Assisted Reproductive Technology (ART). In Australia, it is illegal to use *in vivo* matured human oocytes for this purpose. We set out to determine if immature oocytes collected during ART cycles, and normally discarded, might be used to produce pESC.

With approval of the NHMRC Licensing Committee, a total of 174 immature oocytes were collected from 68 women attending Fertility East for the purpose of ART over 2 years. The oocytes were matured *in vitro* with a 79% success rate, and these then activated with 72% efficiency. Most of these parthenotes (64%) developed into at least 2 cells, 43% into 8 cells, and 10% reached the morula stage; none became blastocysts. Accordingly, pESC could not be created. The arrested development of parthenotes produced from immature eggs that are normally discarded suggests the sub-optimal quality of these eggs.

Next, we examined the availability and usefulness of immature eggs from ovaries being surgically removed to reduce the likelihood of cancer developing in genetically predisposed women ≤ 40 years. 51 immature oocytes were collected from 4 women over a period of 4 years. 49% of the eggs were matured but none became activated.

We conclude that the creation of pESC is currently not possible in Australia. Paradoxically, pESC created from mature oocytes collected in parts of the world where this is permissible, are being used in Australia for the treatment of Parkinson's Disease.

P41 | SHRNA SCREEN FOR NOVEL EPIG ENETIC REGULATORS OF Γ-GLOBIN SILENCING

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A greater understanding of the epigenetic processes that contribute to γ-globin silencing will ultimately help inform the development of therapeutic strategies for the reactivation of γ-globin. In this study, we generated a murine erythroleukaemia cell line that carries an intact 183kb human β-globin locus modified to express eGFP under the control of the γ-globin promoter (GG-MEL). The GG-MEL reporter was used to screen a retroviral shRNA library containing 1027 shRNAs targeting 158 genes encoding epigenetic regulators. Following retroviral transduction, GFP-positive and GFP-negative samples were collected over the course of differentiation. Next generation sequencing and bioinformatics analysis were used to compare differences in shRNA representation between the GFP-positive and GFP-negative samples. The screen identified several genes that are known regulators of γ-globin silencing, as well as genes that have not previously been associated with γ-globin regulation. Following validation of novel candidates, *Kdm2b* emerged as the top regulator of γ-globin silencing. Molecular characterisation was conducted in parental MEL cells and included RNA-seq to investigate global changes in gene expression upon *Kdm2b* knockdown, and ChIP-seq to identify global binding sites of KDM2B. ChIP-seq analysis revealed strong enrichment of KDM2B at actively expressed β-globin genes, but was absent at the developmentally silenced embryonic/fetal globin genes. Knockdown of *Kdm2b* resulted in increased expression of the γ-globin reporter in primary erythroid cells derived from the GG murine fetal livers. These results suggest *Kdm2b* may be an activator of β-globin gene expression and down regulates γ-globin through a competitive model.

P42 | *IN VITRO* RECONSTRUCTION OF ADENOVIRUS-LIKE GENOME: A NOVEL PLATFORM FOR GENE DELIVERY

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In vivo gene therapy relies on efficient delivery of DNA into the nucleus. Although viral vectors deliver DNA payloads efficiently, they suffer from multiple safety and payload drawbacks. Adenovirus (Adv) genomes have a single copy of terminal protein (TP) covalently coupled to the 5' end of each DNA strand. TP role in viral gene delivery across the nuclear pore is completely unexplored. Here, we construct synthetic TP-DNA conjugates *in vitro* and evaluate their potential to improve the efficiency of non-viral gene delivery. We expressed and purified TP with mutated cysteine for downstream sulfhydryl bio-conjugations. TP fusion was further tested for activity using Alphascreen protein interaction assay and microinjection in Hela. TP was conjugated to an oligonucleotides using Sulfo-SMCC activation for subsequent linking to DNA. TP-TRx was expressed and accumulated as an insoluble fraction. After solubilisation, TP was refolded on column. Refolded protein labelled with AF-594 readily accumulated in the nucleus, validated by co-injection of a cytoplasmic marker. TP interacted strongly with importin β or αβ, but not importin α, complex with K_d of 21.17 or 58.83 nM, respectively. TP in both cleaved or non-cleaved form (TP-TRx) can be successfully conjugated to DNA oligonucleotides using Oligo-Maleimide:TP-cysteine conjugation ratios of 2:1 or above. These results demonstrate that TP is functional after expression in bacteria and refolding. In addition, the TP can be conjugated directly to oligonucleotides for further ligating to a linear DNA payload. Current and future works focus on studying the delivery kinetics of TP-conjugated DNA across the nucleus.

P43 | INDUCED PLURIPOTENT STEM CELLS FROM A MARSUPIAL, THE TASMANIAN DEVIL (*SARCOPHILUS HARRISII*): INSIGHT INTO THE EVOLUTION OF MAMMALIAN PLURIPOTENCY

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The study of marsupial biology has both intrinsic and comparative significance, and may serve to stem the further loss of marsupial diversity due to extinction of species. Induced pluripotent stem cells (iPSCs) are adult somatic cells that have been re-programmed to an embryonic stem cell-like state, providing a powerful research tool. We demonstrate the generation of Tasmanian devil (*Sarcophilus harrisii*) iPSCs (DeviPSCs), the first marsupial stem cells, from dermal fibroblasts by lentiviral transduction of human transcription factors: *OCT4*, *SOX2*, *KLF4*, *NANOG*, *c-MYC* and *LIN-28*. DeviPSCs display characteristic pluripotent stem cell colony morphology, with individual cells having a high nuclear to cytoplasmic ratio and alkaline phosphatase (AP) activity. DeviPSCs are leukemia inhibitory factor (LIF) dependent and have reactivated endogenous *POU5F1* (*OCT4*), *POU5F3* (*POU2*), *SOX2*, *NANOG* and *DAX1* genes, retained a normal karyotype, and concurrently silenced exogenous human transgenes. Notably, expression of both *POU5F1* and *POU5F3* appears to reflect a naive state of pluripotency since both factors are known to be expressed by cells from the epiblast of marsupial pre-implantation embryos. Under culture conditions favouring differentiation, DeviPSCs readily formed embryoid bodies (EBs) and *in vitro* teratomas containing derivatives of all three embryonic germ layers. To date, DeviPSCs have been stably maintained for more than 45 passages. Thus, our DeviPSCs are important in understanding marsupial biology and the evolution of mammalian pluripotency. Significantly, these DeviPSCs will be an indispensable asset for stem cell-based regenerative and anti-cancer therapies against Devil Facial Tumour Disease (DFTD) which has threatened Tasmanian devils with extinction.

Funding: This study was supported by a grant awarded to Dr. Deanne Whitworth by Morris Animal Foundation, United States and Australian Government Research Training Program Scholarship offered to Prasanna Weeratunga.

P44 | ANTISENSE OLIGONUCLEOTIDE TREATMENT OF *COL7A1* CAUSES NON-SPECIFIC SPLICE MODIFICATIONS

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Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited disease caused by bi-allelic mutations in the *COL7A1*, which results in severe blistering of the skin and mucous membranes. Current therapies treat the symptoms but not the disease. Most mutations are either small indels, point mutations that disrupt the folding of the Gly-X-Y repeats in the collagenous zone, or null mutations causing premature termination of the protein. Of the 118 exons in *COL7A1*, 107 are in-frame and removal of one of these would not disrupt the mRNA reading frame. Therefore, using antisense oligonucleotides (AO) to alter splicing and remove these mutations could be a potential therapy to

treat RDEB. Natural exon skipping removing a nonsense mutation in *COL7A1* exon 15 and a single base-pair insertion in exon 19 resulted in modest increases in functional COL7A1 expression and reduced disease severity in both RDEB patients. Hence, the removal of selected individual exons does not seem to affect the trimerisation of COL7A1, and therefore preserves the fibril function. However, since most *COL7A1* exons are separated by small introns, targeting a single exon could impact splicing of adjacent exons. We have found that targeting *COL7A1* exon 73 induced robust skipping of exon 73, but also caused removal of exon 74 and retention of intron 76. Further investigation is being carried out to understand and eliminate these non-specific splice events. Restricting the range for transcript analysis, especially using nucleic acid based assays, could fail to detect the overall effectiveness and specificity of AOs to splice-switch accordingly.

P45 | RNA-SEQUENCING TO DISCOVER NOVEL LONG-NON CODING RNA (LNCRNA) ASSOCIATED WITH OBESITY IN RAT LIVER

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Obesity rates are increasing in Australia and world-wide leading to an increase in obesity-related diseases such as diabetes, cardiovascular disease and some cancers. In addition to lifestyle and pharmacological approaches, gene therapy is an area of current interest for the treatment of metabolic diseases such as obesity. Sense/antisense pairs of protein coding gene/lncRNA are an attractive target for manipulation. This is because they give the option of either direct reduction of sense transcript or an indirect increase of the sense by reducing the antisense lncRNA transcript. High fat diet feeding for 21 weeks generated obese rats that were on average 34% heavier than controls. We screened the liver transcriptomes of 4 rats from each group for novel lncRNA that were increased or decreased by obesity, and are antisense to protein-coding genes. Here we present our initial results. We discovered 4 novel sense/antisense pairs, at *Il7*, *Acot4*, *Gadd45a* and *Cyp8b1*. As IL-7 is markedly increased in the serum of obese individuals we are currently focussing our *in vitro* experiments on the lncRNA that is antisense to the interleukin-7 gene.

P46 | MODELING HUMAN METABOLIC LIVER DISEASE IN FRG MICE BY ENGRAFTMENT AND EXPANSION OF PATIENT-DERIVED HEPATOCYTES: TOWARDS AAV-MEDIATED GENE THERAPY

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The liver is an immensely promising gene therapy target, with success in the treatment of haemophilia B using recombinant AAV (Nathwani *et al*., NEJM, 2014) underscoring the broader potential of this approach. Notably, however, therapeutic benefit in haemophilia B is achievable with hepatocyte transduction efficiencies of ≤3%. By contrast, the majority of therapeutically amenable genetic/metabolic liver diseases will require considerably higher transduction efficiencies that are beyond the reach of existing AAV technology. A major challenge in further developing AAV-based technology is that gene transfer efficiencies vary in a species-dependent manner, with animal data not predicting human performance. *In vitro* studies of primary human hepatocytes are also precluded by culture-induced changes in differentiation state. To address these challenges we have exploited the FRG mouse model ($FAH^{-/-}$/$Rag2^{-/-}$/$IL2rg^{-/-}$), bearing chimeric mouse-human livers, established by engraftment and selective expansion of primary human hepatocytes obtained from healthy and urea cycle-deficient livers. In earlier collaborative studies we have successfully used this model to screen shuffled capsid libraries to select capsid variants capable of transducing primary human hepatocytes 1-2 orders of magnitude more efficiently than the best available clinically tested capsids (Lisowski *et al*., Nature 2014). In the current study we are extending the utility of the FRG mouse model by demonstrating that mice, highly engrafted with urea cycle-deficient hepatocytes, develop metabolic disturbances characteristic of the human disease. Once fully validated, this model system will allow direct assessment of the capacity of clinically configured AAV vectors to correct the metabolic phenotype of primary human hepatocytes *in vivo*.

P47 | HUMAN AMNION EPITHELIAL CELL-DERIVED EXTRACELLULAR VESICLES IN THE TREATMENT OF BRONCHOPULMONARY DYSPLASIA

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We have previously showed that human amnion epithelial cells (hAECs) can be a viable source of cell therapy for established bronchopulmonary dysplasia (BPD), and extracellular vesicles (EVs)

derived from pro-regenerative cells could be a potential remedy by transporting proteins, lipids, mRNA and microRNA. Here we characterized hAEC-derived EVs and assessed their efficacy on neonatal lung injury where BPD-like injury was achieved using a combination of antenatal inflammation and postnatal hyperoxia. The isolated hAEC-EVs had distinct cup shaped morphology with average size of 40-120nm. ALIX (96kDa), Grp94 (92kDa) and HLA-G (38kDa) were expressed in EVs and Pathway enrichment analysis showed that endothelin signaling pathway, Wnt signaling pathway, and inflammation mediated by chemokine and cytokine signaling pathway were enriched in hAEC-EVs. In mouse model of BPD-like lung injury, we observed that hAEC-EVs improved tissue-to-airspace ratio and septal crest density in a dose-dependent manner. hAEC-EV administration reduced the levels of inflammatory cytokines such as interleukin (IL)-1β and tumour necrosis factor-alpha (TNF-α). The improvement of lung injury was associated with the increase of the percentage of type II alveolar cells. Surprisingly, neonatal hAEC-EV delivery reduced airway hyper-responsiveness, mitigated pulmonary hypertension and prevented right ventricle hypertrophy that associated with BPD-like lung injury, and this persisted until to 10 weeks of age. These findings suggest that hAEC-derived EVs can be a potential cell-free therapy for babies at risk of developing BPD.