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ABSTRACT

NINTH AUSTRALASIAN GENE AND CELL THERAPY SOCIETY MEETING

Date

April 29 – May 1 2015

Venue

University College,
The University of Melbourne,
Parkville, Victoria, Australia

The Australasian Gene and Cell Therapy Society (AGCTS) held its Ninth Biennial Meeting between 29 April and 1 May 2015. The AGCTS 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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Gene Therapy Research Unit,
Children's Medical Research Institute and
The Children's Hospital at Westmead,
Westmead, NSW, Australia

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KEYNOTE PRESENTATIONS

KEYNOTE SPEAKER 1: LUNG CANCER: MICROTUBULES AND THERAPEUTIC TARGETING

Sponsored by Benitec BioPharma Ltd

Maria Kavallaris[1,2], [1]Children's Cancer Institute, Lowy Cancer Research Centre, UNSW, Randwick, NSW, Australia, [2]ARC Centre of Excellence for Convergent Bio-Nano Science and Technology, Australian Centre for NanoMedicine, UNSW, NSW, Australia

Lung cancer remains the most common cause of cancer-related death in the world. The most common form, nonsmall cell lung cancer (NSCLC), is often diagnosed at advanced stage disease and overall 5-year survival rates for this group are dismal. Chemotherapeutic treatments for NSCLC often involve a combination of a platinum-based drug (e.g. cisplatin or carboplatin) and a tubulin-binding agent (e.g. taxol or vinorelbine). Unfortunately, drug resistance remains a major clinical problem in NSCLC. Expression of βIII-tubulin (encoded by *TUBB3* gene) is associated with resistance to tubulin-binding agents in a range of tumour types including NSCLC [1]. We identified a broad role for βIII-tubulin in chemosensitivity by showing that it can mediate response not only to tubulin-binding agents, but also to broad classes of drugs, including DNA damaging agents in NSCLC [2,3]. Moreover, we recently described a multifactorial role for *TUBB3*/βIII-tubulin in tumourigenesis and metastasis where it influences a major cell signaling survival pathway [4]. Collectively, these and other studies have validated βIII-tubulin as a therapeutic target in NSCLC. Due to the homology of the various β-tubulin isotypes, specific targeting with small molecule inhibitors has remained a challenge. To overcome this, we have been developing gene silencing approaches against cytoskeletal targets. One of our approaches has been the design and use of DNA-directed RNA interference (ddRNAi) to silence *TUBB3*/βIII-tubulin. We have achieved potent *in vivo* suppression of the *TUBB3* gene in an orthotopic model of NSCLC. Importantly, combining suppression of *TUBB3* and chemotherapy led to a significant increase in mouse survival rates compared to control animals. Effective therapies for NSCLC are urgently required and an increased understanding of resistance mechanisms and the development of new approaches to therapeutics has the potential to improve survival rates for this aggressive malignancy.

1. Kavallaris M. Microtubules and resistance to tubulin-binding agents. *Nat Rev Cancer* 2010; **10**: 194–204.
2. McCarroll JA, Gan PP, Liu M, Kavallaris M. betaIII-tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. *Cancer Res* 2010; **70**: 4995–5003.
3. Gan PP, Pasquier E, Kavallaris M. Class III beta-tubulin mediates sensitivity to chemotherapeutic drugs in non small cell lung cancer. *Cancer Res* 2007; **67**: 9356–9363.
4. McCarroll JA, Gan PP, Erlich RB, *et al*. TUBB3/betaIII-tubulin acts through the PTEN/AKT signaling axis to promote tumorigenesis and anoikis resistance in non-small cell lung cancer. *Cancer Res* 2015; **75**: 415–425.

KEYNOTE SPEAKER 2: TT-034: A ddRNAI-BASED VECTOR USED FOR THE TREATMENT OF INDIVIDUALS INFECTED WITH THE HEPATITIS C VIRUS

Sponsored by Benitec BioPharma Ltd

David Suhy Sr VP Research & Development, Benitec Biopharma

Over 170 million individuals worldwide are living with chronic hepatitis C, and infection resulting from infection with the hepatitis C virus (HCV). Despite the recent addition of more effective DAAs, the current standard of care is expensive, requires daily dosing for up to 24 weeks and, in most cases, must be used in combination of drugs with significant side effects. By contrast, the use of ddRNAI (DNA-directed RNA interference) based drugs offers the possibility of a 'one-shot-cure', where the disease is treated with a single infusion. Because the viral genome is comprised of a single strand of RNA and its replication occurs strictly within the cytoplasm, HCV is an ideal candidate for therapeutics based upon RNAi. Benitec Biopharma is in the midst of a first in man, phase I/IIa open label dose escalating clinical study in which subjects with chronic HCV infection are treated with an RNAi-based therapeutic, termed TT-034. This agent contains a single recombinant genome to express three independent shRNAs that simultaneously target multiple well-conserved regions of the HCV genome. TT-034 uses an adeno-associated virus capsid to deliver the agent into hepatocytes following intravenous administration, with almost 100% of hepatocytes being transduced at clinically relevant doses. In addition, pre-clinical studies in mice and nonhuman primates have demonstrated persistent expression of shRNA out to 180 days, the duration of each of the studies. To date, human subjects in the first two cohorts have been administered doses of TT-034 at either 4e10 or 1.25e11 vg/kg. Because these low doses only transduce a fraction of the hepatocytes, the principal readout from the first cohorts has been safety as well as the level of TT-034 liver transduction, as assessed from liver biopsies taken 3 weeks after dosing. The level of transduction found in these biopsies is consistent with data obtained from the nonhuman primate models. Furthermore, there have been no treatment-related serious adverse events in the study to date. In summary, we find that subtherapeutic doses of TT-034 appear to be well tolerated in human subjects infected with HCV. The trial will continue to progress into the higher dose cohorts with the aim of achieving a significant and robust impact on the hepatitis C viral load without significant safety effects.

KEYNOTE SPEAKER 3: DIRECTED EVOLUTION OF NEW ADENO-ASSOCIATED VIRUSES FOR THERAPEUTIC GENE DELIVERY

Sponsored by Benitec BioPharma Ltd

David Schaffer[1,2], [1]University of California, Berkeley, CA, USA, [2]4D Molecular Therapeutics, San Francisco, CA, USA

Strong basic and translational efforts in the gene therapy field have culminated in successes in an increasing number of human trials involving adeno-associated virus (AAV) vectors. These studies firmly establish that AAV is capable of safe and therapeutic gene delivery to some targets, although it is also apparent that vectors based on natural versions of AAV in general face a number of challenges that hinder the extension of these successes to most human diseases. These include the prevalence of neutralizing antibodies against natural AAV serotypes in the human population, limited biodistribution to many target tissues, poor spread into these tissues, an inability to target delivery to specific cells, and low transduction efficiency of many therapeutically relevant cells. These challenges are not surprising, as nature did not evolve viruses for our convenience to use as human therapeutics. Over the past 15 years or more, we have developed and implemented directed vector evolution to create novel, optimized vectors for a range of clinical applications. Directed evolution, which emulates the natural evolution process, is the iterative genetic diversification and functional selection of biomolecules for desired properties.

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In the case of AAV, we have created large ($> 10^8$) libraries of AAV capsid variants and implemented functional selections to isolate 'designer viruses' with delivery properties that are substantially better than natural AAV serotypes. For example, it would be desirable to develop non-invasive means to deliver genes to the retina; however, natural AAV variants require an invasive subretinal injection, which can further damage an already degenerating tissue, to transduce target cells involved in most retinal degenerations (photoreceptors and retinal pigment epithelium). We have evolved AAV to mediate high efficiency gene delivery to photoreceptors upon a simple injection into the vitreous humor of the eye. We have also applied directed evolution to create vector variants that evade human neutralizing antibodies, mediate gene delivery and gene targeting in stem cells, and undergo enhanced transport in the central nervous system, among other properties. In summary, continuing the process of AAV evolution, but changing the direction towards functions and properties that are optimized for biomedical application, is a promising approach to create designer vectors for human gene therapy.

KEYNOTE SPEAKER 4: COMBINING GENE THERAPY AND GENE SILENCING FOR TRANSFORMATIONAL THERAPEUTICS

Sponsored by Benitec BioPharma Ltd

Peter French CEO and Managing Director, Benitec Biopharma Ltd

DNA-directed RNA (ddRNAi) interference is a technology that provides the ability to produce permanent gene silencing by delivering DNA constructs to cells and tissues. The DNA constructs continuously produce shRNA for the lifetime of the cell, allowing for single administration treatments and cures for a range of human disease. Benitec is developing ddRNAi for hepatitis C (currently in the clinic), hepatitis B, nonsmall cell lung cancer, wet age-related macular degeneration and the orphan genetic disease oculopharyngeal muscular dystrophy. This presentation will address how combining the flexibility of gene therapy vectors with the power and specificity of expressed shRNA is leading to novel therapies for serious, life threatening major diseases.

THE GREG JOHNSON MEMORIAL ORATION

KEYNOTE SPEAKER 5: REGULATION OF PI3K/AKT-DEPENDENT BREAST CANCER GROWTH AND METASTASIS

Lisa M. Ooms[1], Lauren C. Binge[1], Parvin Rahman[1], James R. Conway[2], Daniel T. Ferguson[1], Clare G. Fedele[1], Rajendra Gurung[1], Jessica L. Vieusseux[1], Ryan C. Chai[1], John T. Price[1], Tony Tiganis[1], Paul Timpson[2], Catriona A. McLean[3], Christina A. Mitchell[1], [1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia, [2]Invasion and Metastasis Group, The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, [3]Department of Anatomical Pathology, Alfred Hospital, Prahran, VIC, Australia

Breast cancer is the most common cancer in women. However, despite advances in treatments metastatic disease remains the leading cause of breast cancer death. The phosphoinositide 3-kinase (PI3K) signalling pathway is frequently hyperactivated in breast cancer and represents a significant target for novel therapies. PI3K-generated PtdIns(3,4,5)P_3 activates Akt, which promotes breast cancer cell proliferation. Akt1, but not Akt2, inhibits cell migration and metastasis. PI3K

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signaling is terminated by phosphoinositide phosphatases such as the tumor suppressor, PTEN, which hydrolyzes PtdIns(3,4,5)P_3 to form PtdIns(4,5)P_2 and thereby suppresses Akt signaling. PTEN loss is observed in 30-40% of sporadic cases of breast cancer associated with hyperactivation of Akt signaling and tumor progression. Interestingly, an alternative pathway for the termination of PI3K signaling is mediated by inositol polyphosphate 5-phosphatases (5-phosphatase), which degrade PtdIns(3,4,5)P_3 to form PtdIns(3,4)P_2, and in turn 4-phosphatases such as INPP4B hydrolyze PtdIns(3,4)P_2 to form PI(3)P. Although we and others have identified INPP4B as a tumor suppressor in breast cancer, there is little evidence that any of the 10 mammalian 5-phosphatases suppresses PI3K/Akt signaling in breast cancer. Here, we identify an inositol polyphosphate 5-phosphatase suppresses oncogenic Akt signaling in breast cancer and shows loss of expression in triple negative breast cancers. Using mouse knockout models and shRNA knockdown in breast cancer cell lines, we demonstrate that loss of the 5-phosphatase leads to enhanced cell proliferation and tumour growth but, paradoxically, suppression of metastasis via regulation of cell migration and invasion. Collectively, these studies identity a novel regulator of PI3-kinase signalling in breast cancer.

KEYNOTE SPEAKER 6: UPDATE ON GENE THERAPY CLINICAL TRIALS FOR THE TREATMENT OF β-HEMOGLOBINOPATHIES

Sponsored by BlueBird Bio, Calimmune, Murdoch Childrens Research Institute and Thalassaemia Australia

Philippe Leboulch[1,2,3] and the LentiGlobin clinical trial study group (by alphabetic order): Y. Beuzard, M. Cavazzana, S. Fucharoen, S. Hacein-Bay Abina, J. Ho, S. Hongeng, J. L. Kwiatkowski, S. Larsen, J. Macpherson, O. Negre, E. Payen, A. Petrusich, J. E. J. Rasko, G. Schiller, S. Soni, A. A. Thompson, C. von Kalle, M. Walters, [1]University of Paris 11 and CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay-aux-Roses, France, [2]Harvard Medical School and Brigham & Women's Hospital, Boston, MA, USA, [3]Mahidol University and Ramathibodi Hospital, Bangkok, Thailand

The β-hemoglobinopathies (β-thalassemia and sickle cell disease) are the most prevalent inherited disorders worldwide and affect millions. Patients with β-thalassemia major cannot survive without monthly, lifelong transfusions together with iron chelation therapy, and severe cases of sickle cell disease suffer from multiple life-threatening complications. Both categories of patients often have a shortened life expectancy in spite of supportive therapies, which impose an enormous financial burden on affected countries. The only available curative therapy is allogeneic hematopoietic stem cell transplantation, although most patients do not have an HLA-matched, geno-identical donor, and those who do still risk graft rejection or graft-versus-host disease. This is why gene therapy, by *ex vivo* transfer into the patient's hematopoietic stem cells of a derivative of the normal β-globin gene whose expression is appropriately regulated, is an attractive novel therapeutic modality. However, gene therapy of these disorders is especially challenging given the requirement for massive hemoglobin production in a lineage specific manner and the lack of selective advantage for corrected hematopoietic progenitors. During the past two decades, we and others have devised suitable gene transfer vectors and applicable protocols to achieve the permanent correction of mouse models of the β-hemoglobinopathies (*Nature* 2000; *Science* 2001). We have then

obtained approval for the first human clinical trial worldwide for the gene therapy of inborn genetic disorders by lentiviral vector. The first treated patient, a French citizen of Thai and Vietnamese descent with severe β^E/β^0-thalassemia dependent on monthly transfusions since early childhood, has become transfusion independent for 6.9 years (last transfusion on 6 June 2008) (*Nature* 2010). A further optimized vector (BB305) with high-grade purification is now being used in subsequent multicenter clinical trials in the USA and in France, both for β-thalassemia and sickle cell disease, sponsored by the biotechnology company bluebird bio. As of the last public disclosure in December 2014, two more β-thalassemia major patients were transplanted and analyzable in France and four in the USA, including an Australian patient. All had severe β^E/β^0-thalassemia, except for one subject who was β^0/β^0. All six patients became rapidly transfusion-independent, including the β^0/β^0, shortly after transplantation with vector-transduced hematopoietic $CD34^+$ cells. They show blood hemoglobin concentrations near normal values. Extension of the ongoing trials and complementary or alternative approaches to increase safety and efficacy will be discussed together with prospects for the gene therapy of sickle cell disease.

KEYNOTE SPEAKER 7: NEW RAAV VECTORS AND APPROACHES FOR EPISOMAL AND INTEGRATION BASED GENE TRANSFER

Mark A. Kay, Departments of Pediatrics and Genetics Stanford University, Stanford, CA, USA

Recombinant AAV vectors (rAAV) have provided some successes in early clinical trials. However, when moving from animals to humans, a number of unanticipated responses have occurred that resulted in lower than expected efficacy. As more human data are obtained, it has become clear that the large differences in vector transduction profiles between various animal species makes it difficult to select an appropriate preclinical model and extrapolate results into humans. We provide an argument for the use of a chimeric murine-human liver xenograft mouse model as a more reliable surrogate to establish clinical outcomes. In addition, we have used human xenotransplant models in combination with multispecies shuffled AAV capsid libraries to select for chimeric capsids that, when vectorized, provide novel transduction properties. Recent capsid isolates as well as new methods for screening will be discussed. The episomal nature of the vector genomes restrict classical rAAV-mediated gene transfer to quiescent tissues. Moreover, even with the low rate of AAV integration, high rates of hepatocellular carcinoma resulting from promoter activation of oncogenic loci in young mice has raised concerns about treating infants. To circumvent these concerns and provide a viable approach to treating infants, we have developed an AAV promoterless gene targeting approach without the use of nucleases to successfully treat mice with hemophilia B. Additional applications and further development of this technology will be discussed.

KEYNOTE SPEAKER 8: *IN VIVO* EXPANSION OF HEPATOCYTES WITH TARGETED RAAV INTEGRATION RESULTS IN A > 100-FOLD INCREASE OF TRANSGENE EXPRESSION

Sponsored by the Children's Medical Research Institute

Markus Grompe, Oregon Stem Cell Center, Oregon Health & Science University, USA

The recent development of promoter-less transgene integration technology using 2A

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fusions promises enhanced safety over traditional gene therapies. These vectors target therapeutic transgenes to loci for highly expressed endogenous genes through homologous recombination. Due to the inherent low frequency of targeted integrations, transgene expression is limited and requires high vector doses. Here, we describe a modified 2A fusion integration vector that facilitates *in vivo* selection and expansion of gene-targeted hepatocytes. In mice treated with this vector, factor IX transgene expression increased over 400-fold during selection. Selection was achieved by incorporating a genetic element into the integration vector that rescues gene targeted hepatocytes from induced hepatotoxic conditions. Blocking Fah (fumarylacetoacetate hydrolase) leads to hepatotoxic accumulation of the enzyme's substrate fumarylacetoacetate (FAA). This toxicity can be rescued by inhibiting 4-hydroxyphenylpyruvate dehydroxygenase (HPD), an enzyme upstream of Fah. In Fah-deficient mice, hepatotoxity can be readily manipulated by administering or withdrawing the drug NTBC [2-(2-nitro-4-trifluoromethylbenzoyl)-1,3 cyclohexanedione]. We have previously shown that Fah-deficiency can also be rescued in these mice by blocking HPD with a shRNA. This HPD-blocking shRNA was incorporated into the original albumin-targeting 2A integration vector by inserting a shRNAmir into albumin intron sequence, just downstream of the albumin stop codon. The resulting AAV-8 vector was administered to Fah-deficient neonates (P0, $n = 8$) by facial vein injection at a dose of 4e11 Vg. At 4 weeks of age, plasma hF-IX levels measured 167 ± 65 ng/ml. The mice were then cycled off NTBC to select for hepatocytes rescued through expression of the HPD shRNAmir. Plasma hF-IX levels increased exponentially, reaching 46 000 ± 5000 ng/ml after 11 weeks of NTBC cycling ($n = 4$). Three of the treated mice were cycled off NTBC for just 3 weeks, then maintained on NTBC for an additional 6 weeks. hF-IX levels remained steady at 400–1100 ng/ml, suggesting the integrated sequences were not toxic to hepatocytes. Control mice treated with the original albumin integration vector (lacking the HPD shRNAmir) showed no increase in plasma hF-IX levels during NTBC cycling. Administration of the selectable AAV to adult mice has also resulted in increased hF-IX during NTBC withdrawal. To demonstrate that this selection system also works in wild-type mice, we injected neonates with the selectable vector and then subjected them to treatment with a pharmacological Fah-inhibitor (CEHPOBA) for 3 weeks after weaning. hF-IX levels increased over 20-fold in CEHPOBA treated wild-type mice and reached therapeutic levels (approximately 1000 ng/mL). By contrast, the hF-IX levels remained unchanged at their subtherapeutic baseline in untreated controls. In summary, 2A fusion-based integration vectors promise significant safety advantages over traditional gene targeting methods. Our data suggest that incorporating selectable genetic elements into such vectors can facilitate highly robust transgene expression which can be modulated *in vivo*. Selectable vectors could also facilitate significantly lower vector doses, thereby enhancing the safety profile of such vectors even further.

KEYNOTE SPEAKER 9: MULTIGENERATIONAL UNDERNUTRITION, INSULIN RESISTANCE AND SUSCEPTIBILITY TO DIABETES

Anandwardhan A. Hardikar, on behalf of the Thrifty Jerry Study Group, NHMRC Clinical Trials Centre, Medical Foundation Building, University of Sydney, Camperdown, NSW, Australia

People in developing countries have faced multigenerational multinutrient undernutrition

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and are currently undergoing major lifestyle changes, contributing to an epidemic of metabolic disease. The underlying mechanisms are unclear. We assessed glucose-insulin metabolism in Wistar rats after 50 generations of undernutrition, and after two generations of unrestricted access to commercial chow (nutrient transition/recuperation). Undernourished rats demonstrated low birth-weight, high visceral adiposity (DXA/MRI) and insulin resistance (hyperinsulinemic-euglycemic clamps) compared to age/gender-matched Control rats. Relative to Controls, Undernourished rats had higher circulating insulin, homocysteine, endotoxin and leptin levels, lower adiponectin, vitamin B_{12} and folate levels, and an eight-fold increased susceptibility to Streptozotocin-induced diabetes. We demonstrate that the metabolic/epigenetic changes observed in Undernourished rats were not reversed after two generations of nutrient recuperation. Control (lean) rats also show microbial dysbiosis (post-recuperation), associated with insulin resistance. As an extension to these studies, our recent data towards understanding the role of gut microbiota in epigenetic regulation of gene expression and influencing the life-long risk of type 2 diabetes are discussed.

KEYNOTE SPEAKER 10: SCREENING FOR AND CHARACTERISING MAMMALIAN EPIGENETIC MODIFIERS

Kelan Chen[1,2], Jiang Hu[3,4], Darcy L. Moore[1,2], Ruijie Liu[1], Kelsey Breslin[1], Natasha Jansz[1,2], Sarah A. Kessans[5], Isabelle S. Lucet[1,2], Andrew Keniry[1,2], Huei San Leong[1,2], Clare L. Parish[6], Douglas J. Hilton[1,2], Richard J. L. F. Lemmers[7], Silvère M. van der Maarel[7], Peter E. Czabotar[1,2], Renwick C. Dobson[5], Matthew E. Ritchie[1,2], Graham F. Kay[3], James M. Murphy[1,2], Marnie E. Blewitt[1,2], [1]WEHI, Melbourne, VIC, Australia, [2]University of Melbourne, Melbourne, VIC, Australia, [3]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia, [4]Queensland University of Technology, Brisbane, QLD, Australia, [5]Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [6]The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia, [7]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

Epigenetic control is of critical importance for normal biology and when disrupted results in disease. We are interested in understanding the molecular mechanisms of epigenetic control, in to allow intelligent drug design for therapeutic manipulation of epigenetic state. However, to understand the mechanisms of control, we must first identify the proteins involved. Therefore, we aim to identify novel epigenetic modifiers or to ascribe new roles to known epigenetic regulators, and characterise the functional and molecular role of these epigenetic modifiers in mitotically heritable epigenetic regulation. One example of such studies is our work on Smchd1, which we identified in a previous *in vivo* mutagenesis screen. We have described the critical role Smchd1 plays in X inactivation, identified a role for Smchd1 in genomic imprinting and regulation of the clustered protocadherins, and our collaborators have now shown that heterozygous *SMCHD1* mutations are responsible for facioscapulohumeral dystrophy type 2 (FSHD2). However, there has been no understanding of how Smchd1 elicits transcriptional silencing. We now report the first high-resolution analysis of the genomic occupancy of Smchd1, which we have partnered with genome-wide analysis of gene expression, histone marks and DNA methylation. In conjunction with our biochemical and biophysical characterisation of Smchd1, these studies have demonstrated that Smchd1 has the capacity to bind directly to DNA, potentially RNA. These studies have informed a new model of Smchd1 action that will enable us to work towards enhancing SMCHD1 function to

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treat FSHD2. This work demonstrates the utility of epigenetic screens to reveal epigenetic modifiers important both for normal development, and with important roles in human disease.

KEYNOTE SPEAKER 11: OPTICAL TOMOGRAPHY AND ITS ROLE IN MULTIMODALITY IMAGING AND TRANSLATIONAL DISEASE RESEARCH

Sponsored by Thermo-Fisher Scientific

Kevin P. Francis, Life Sciences, PerkinElmer, Hopkinton, MA, USA

PerkinElmer is a global leader in the development of instrumentation and probes for small animal non-invasive imaging, including optical, PET and μCT imaging. Through optical imaging, we have developed a technology which allows biological processes, including gene expression that is temporally and spatially defined, to be non-invasively monitored both longitudinally and in real-time. Genes encoding optical reporters, luciferases and fluorescent proteins, are engineered into cells (e.g. cancer cells, stem cells) and pathogens (e.g. bacteria, viruses), or directly into animals (e.g. monitoring host responses) to enable the generation of light that can be visualized through the tissues of a live animal. PerkinElmer is the only company to have optimized this technique to allow true three-dimensional optical imaging and tomographic multimodality imaging (e.g. through co-registration of optical imaging with μCT and MRI). Furthermore, this technique is equally applicable to imaging of fluorescent dyes and particles, allowing fluorescently tagged biological events (e.g., tracking of antibodies, peptides and viral capsids) to be monitored both independently and in combination with genetically tagged events. PerkinElmer has also recognized the importance of moving optical imaging beyond monitoring small animals, allowing its fluorescent probes to be seamlessly translated from preclinical to diagnostic and surgical applications in large animals (e.g. veterinary), with the view of eventually moving this technology into routine clinical settings. An overview of optical imaging in a range of disease backgrounds will be presented, showing how this approach can be used to refine and improve fundamental biological research, as well as drug development strategies and surgical procedures.

KEYNOTE SPEAKER 12: TRANSCRIPTIONAL REPROGRAMMING AND DIRECTED DIFFENRENTIATION OF PLURIPOTENT STEM CELLS: ALTERNATIVE OPTIONS FOR REGENERATING KIDNEY

M. H. Little[1,2,3], J. M. Vanslambrouck[2], M. Takasato[1,2], J. Li[2], N. Suhaimi[1,2], P. X. Er[1,2], H. Chiu[2], [1]Murdoch Children's Research Institute, Royal Children's Hospital, Australia, [2]Institute for Molecular Bioscience, University of Queensland, QLD, Australia, [3]Department of Pediatrics, University of Melbourne, Melbourne, VIC, Australia

As chronic kidney disease incidence increases, so does the need for novel therapeutics. Because all the nephrons in the human kidney arise prior to birth, regeneration of complete nephrons may require the recreation of embryonic nephron progenitors (NPs). We have taken two approaches to this challenge; directed differentiation of human pluripotent stem cells to kidney progenitors and direct transcriptional reprogramming of somatic cells to nephron progenitor. Using a lentivirus-mediated screen, we previously identified six developmental transcription factors (*SIX1, SIX2, HOXA11*, *OSR1, EYA1* and *SNAI2)* sufficient to re-impose an NP-like phenotype when co-expressed in adult human proximal tubule cells (Hendry *et al*., *JASN*

2013). These transcription factors have now been moved into a hyperactive *piggyBac* transposase (m7pB) system for Doxycycline-mediated inducible expression coupled with the expression of mCherry. Using this approach, we have generated stable HK2 cell lines and have demonstrated the capacity to reprogram such cells to a nephron progenitor state which, once relaxed, results in the *in vivo* generation of new nephrons in a neonatal animal model. Using our understanding of the embryological basis of kidney organogenesis, we have also developed culture protocols for the stepwise induction of posterior primitive streak, anterior and posterior intermediate mesoderm and subsequently progenitors for both the formation of nephrons and the ureteric epithelium required to create a kidney. Such cultures, when aggregated and grown in 3D, act as a self-organising organoid generating numerous patterned and segmented nephrons connected to a collecting duct as would occur during normal embryogenesis. Each of these approaches provides novel opportunities for cellular therapy, tissue regeneration and disease modelling.

ORAL PRESENTATIONS

O1: DISCOVERY OF ANCIENT AND CONTEMPORARY ADENO-ASSOCIATED VIRUSES FROM AUSTRALIAN MARSUPIALS

Claus V. Hallwirth[1], Richard H. Smith[2], Nicola A. Hetherington[1], Mona-Larissa Ziegler[1], Michael Westerman[3] Robert M. Kotin[2], Ian E. Alexander[1,4], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, NSW, Australia, [2]Laboratory of Molecular Virology and Gene Therapy, National Heart, Lung and Blood Institute, Bethesda, MD, USA, [3]Department of Biochemistry and Genetics, La Trobe University, Bundoora, VIC, Australia, [4]The University of Sydney, Discipline of Paediatrics and Child Health, Westmead, NSW, Australia

Recombinant adeno-associated viruses (rAAVs) are proving to be powerful tools for genetic manipulation in the context of gene therapy and basic research. The capacity to pseudo-serotype the prototypic AAV2 genome with capsids from other AAV serotypes of nonhuman origin has greatly broadened the range of tissues and cell types that can be efficiently transduced using rAAV, although there remain significant gaps in the transduction repertoire. Given the evolutionary isolation of Australian marsupials, we reasoned that novel marsupial AAV isolates could provide an invaluable resource for further development of the rAAV system. Using the polymerase chain reaction to target sequences conserved among known AAV isolates, we screened DNA samples obtained from an eastern grey kangaroo. This led to the detection of a novel AAV-derived sequence. Further analysis of other marsupial species revealed that we had identified an endogenous viral element (EVE) representative of an AAV that had become fixed in the ancestral genome of all Macropodiformes (marsupials characterised by bipedal hopping locomotion) between 45 and 27 million years ago. We have also detected the first-ever contemporary marsupial AAVs, showing a higher degree of homology to the ancient AAV-EVE than to any known contemporary AAV, in a range of marsupial faecal samples. Collectively, these data suggest that the AAV genome has remained remarkably stable through evolutionary time and that AAVs will prove to be widespread in marsupials. Additionally, AAV-EVEs represent an untapped resource for the resurrection of novel AAV capsid sequences with the potential to complement the identification of novel capsids from contemporary marsupial AAVs.

O2: DEVELOPMENT OF SYNTHETIC DNA REFERENCE MATERIAl FOR ACCURATE ANALYSIS OF GENE THERAPY VECTORS

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Accurate and reliable analysis of gene therapy vectors is required in establishing dosage and in biodistribution studies. It is also crucial in the detection of gene doping, the illegal misuse of gene therapy in sport. Vector genomes are typically analysed by quantitative polymerase chain reaction (PCR) using laboratory calibrants based on a plasmid incorporating transgenic sequences. These calibrants often undergo limited characterisation, differ between manufacturers and may vary between lots. This could lead to inconsistent results between laboratories and affect clinical outcomes. Importantly, inadvertent contamination of negative samples with such calibrants could lead to false positive results. We developed a unique design strategy for synthetic DNA reference materials (RMs) that contain modified transgenic sequences with the intention of preventing false positive results due to cross-contamination. When such a RM is amplified in transgene-specific assays, the amplicons are distinguishable from those of the vector because they differ in size and sequence. Using human erythropoietin as a model, we designed and produced a RM according to this strategy. To improve accuracy and reliability of vector analysis, the RM was characterised according to ISO Guide 35. Using nonviral and viral vectors carrying erythropoietin transgene, we validated the effectiveness of this RM in vector genome analysis in blood *in vitro*. The developed strategy for RM design could be applied to the generation of RMs for the analysis of other transgenes, genes or transcripts. Together with optimised and validated PCR assays, such RMs form a measurement system that facilitates standardised, accurate and reliable genetic analysis in various applications.

O3: TARGETED DELIVERY OF A SYNTHETIC MICRORNA MIMIC AS AN APPROACH TO CANCER TREATMENT

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MicroRNA expression is commonly downregulated in cancer, contributing to many of the hallmarks of cancer. Recently we demonstrated that multiple members of the miR-15/107 family are downregulated and function as tumor suppressors in malignant pleural mesothelioma (MPM). Using mimics to restore levels of miR-15a, -15b or -16 led to growth inhibition and induction of apoptosis of MPM cells *in vitro*. The miR-16 mimic, packaged in bacterially-derived, EGFR antibody-targeted, EDV™ nanocells (EDVs), inhibited xenograft tumor growth *in vivo*. Because multiple microRNAs from the miR-15/107 family are downregulated in MPM, we investigated whether a single synthetic mimic based on the consensus sequence of the entire family could restore the lost tumour suppressor activity. Four novel mimics derived from the consensus sequence were tested, and compared with a native miR-16 mimic. The novel mimics had enhanced growth inhibitory activity in MPM, nonsmall cell lung cancer (NSCLC) and prostate cancer lines, three tumor types in which miR-15/107 expression is suppressed. They

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were also active in cell lines derived from breast and colon cancer, and sensitised all lines to the antimetabolite nucleoside analog gemcitabine, a mainstay of cancer chemotherapy used to treat many tumour types. Finally, when packaged in EDVs, synthetic mimics also inhibited growth of MPM xenografts *in vivo*. Based on these preclinical studies, a Phase I clinical trial is currently underway for patients with MPM or NSCLC failing standard therapy. This represents only the second trial of microRNA replacement as a cancer therapy, and the first for thoracic cancer.

O4: RNA TARGETING PROMOTER REGION INHIBITS ACTIVATION OF LATENTLY INFECTED HIV-1

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We have been reporting that certain RNA targeting the HIV-1 promoter region can induce transcriptional gene silencing (TGS), which is associated with heterochromatin formation in the HIV-1 promoter region to suppress transcription. We investigate whether or not the RNA-based TGS approach is able to inhibit HIV-1 activation in latently infected cells. We used a lentiviral delivery vector to express shRNA homologous to a conserved sequence in the HIV promoter of HIV-1 (shPromA), a 2-based mismatched variant (shPromA-M2) and scrambled (shPromA-Sc) controls. HIV-1 latent infected U1 cells were transduced with the vectors. These transduced U1 cells were then cultured in the presence of various stimuli, proinflammatory cytokine (TNF-α), histone deacetylase inhibitor (TSA) and hematopoietic growth factor (GM-CSF) for 3 days. A reverse transcriptase (RT) assay detected HIV released from the original U1 cells in the presence of TNF-α. A more than 3-log reduction of RT level was observed in culture supernatant of shPromA transduced-U1 cells, whereas shPromA-M2 or shPromA-Sc transduced-U1 cells failed to suppress HIV-1 production. Similarly the shPromA transduced-U1 cells inhibited production of HIV-1 in the presence of other stimuli. Chromatin-immunoprecipitation assay revealed that the HIV-1 promoter sustained latent phase of heterochromatin in the U1 cells, even in the presence of the strong stimuli. The induced suppression was sequence specific. The shPromA appears to act enhance heterochromatin structure to prevent from shifting to activated phase to sustain closed form of HIV-1 promoter region. TGS approach mediated by the RNA targeting HIV-1 promoter region will be useful strategy to sustain HIV latency.

O5: LIMITING THYMIC PRECURSOR SUPPLY INCREASES THE RISK OF LYMPHOID MALIGNANCY IN SCID-XI MICE: IMPLICATIONS FOR GENE THERAPY PROTOCOLS TARGETING THE HAEMATOPOIETIC COMPARTMENT

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Successful phenotypic correction and immune reconstitution has been achieved in gene therapy trials for X-linked severe combined immunodeficiency (SCID-XI) but leukaemia resulting from insertional mutagenesis has been observed. We previously investigated the utility of lentivirus-mediated correction of a murine SCID-X1 model that phenocopied the human trials. We observed lymphoid malignancies that correlated with low numbers of transplanted progenitors and failed to detect insertional mutagenesis events in the tumours. Recently, the existence of a self-renewing thymic population was revealed in mice in the absence of thymic input [1]. It is considered that acquisition of a self-renewal phenotype and prolonged dwell-time in the thymus increases the risk of gaining genetic lesions. Therefore, by reconstituting our SCID-X1 mice with low frequencies of lentiviral-transduced progenitor cells, we could have inadvertently recapitulated a setting of low thymic input, thereby increasing oncogenic risk. In the present study, we test this hypothesis by limiting the numbers of wild-type bone marrow cells used to reconstitute $RAG2^{-/-}\gamma c^{-/-}$ mice in the complete absence of gene transfer. Limiting thymic precursor supply increased the risk of malignancy and gave rise to lymphomas expressing T cell markers in which activating *Notch1* mutations were readily identified. We propose a conceptual framework to explain why T-ALL is observed in SCID-XI but not in ADA-SCID patients, despite both groups harbouring integrations in the proto-oncogene *LMO2* with the potential to increase thymic dwell-time.

1. Martins VC, Busch K, Juraeva D, *et al*. Cell competition is a tumour suppressor mechanism in the thymus. *Nature* 2014; **509**: 465–470.

O6: GENETICALLY MODIFIED TUMOUR CELLS EXPRESSING IL-15 AND IL-15Rα AS AN *IN SITU* CANCER VACCINATION PLATFORM

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Interleukin-15 (IL-15) is a pleiotropic cytokine with the ability to increase the effectiveness of vaccines through enhancement of both innate and adaptive immune responses. Co-expression of its receptor, IL-15Rα, additionally enhances the biological activity of IL-15 through improved trans-presentation of IL-15 to the signalling ß and common γ receptors and by localising it to the surface of the cells. Using these characteristics, we developed an *in situ* cellular vaccine by genetically engineering tumor cells to express both IL-15 and IL-15Rα, thereby allowing the direct activation of effector cells. To do this, we constructed E1, E3 deleted adenoviruses expressing murine IL-15 and IL-15Rα and transduced murine cancer cells *in vitro* and *in vivo*. We demonstrated that these cells were able to express functional IL-15. When subcutaneously implanted into mice, we found that cells expressing of IL-15 and IL-15Rα significantly impaired or prevented tumor growth compared to Ad.null modified cells ($p < 0.05$). Similarly, when we transduced actively growing tumours in mice with Ad.

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IL-15 and IL-15Rα, we showed that the growth of these tumours was significantly inhibited. The expression of IL-15 and IL-15Rα induced the infiltration of both NK and $CD8^+$ cells into the tumours and generated a tumour-specific cytotoxic T-lymphocyte population capable of tumour lysis and IFN-γ release in *ex vivo* assays. Further, we showed that *in situ* vaccination with IL-15 and IL-15Rα could effectively inhibit the growth of distant tumors without off target effects, thus providing a solid rationale for further investigations of this type of *in situ* vaccine platform.

O7: COHERENCE ANALYSIS DISCRIMINATES BETWEEN RETROVIRAL INTEGRATION PATTERNS IN $CD34^+$ CELLS TRANSDUCED UNDER DIFFERING CLINICAL TRIAL CONDITIONS

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The unequivocal success of several gene therapy trials targeting the haematopoietic system using γ-retroviral vectors was accompanied by instances of insertional mutagenesis that led to the development of leukaemia in some patients. These events motivated the ongoing development of putatively safer integrating vector systems and analysis methods to characterise and compare integration site distributions in cells recovered from treated patients or those experimentally transduced under conditions of potential relevance to clinical trials. The available bioinformatic tools to compare integration datasets focus on the association of integration sites with selected genomic and epigenetic features, and the choice of these features determines the ability to discriminate between datasets. We describe the scalable application of point-process coherence analysis to compare patterns produced by vector integration sites across genomic intervals, uncoupled from association with genomic features. To explore the utility of coherence analysis in the context of an unresolved question, we investigated whether the differing transduction conditions used in the initial Paris and London SCID-X1 gene therapy trials result in divergent genome-wide integration profiles. This could plausibly underlie the apparent disparity in the incidence of leukaemia observed in these trials. Coherence analysis was shown to resolve integration site pattern differences resulting solely from the use of different transduction conditions. Existence of differences in integration site distributions, some of which support the hypothesis that Paris transduction conditions lead to integration behaviour carrying a higher risk of oncogenesis than the London trial conditions, was confirmed by the application of established methods to compare integration datasets.

O8: ANALYSIS OF THE MECHANISMS UNDERLYING THE DEVELOPMENTAL REGULATION OF EMBRYONIC AND FETAL β-LIKE GLOBIN GENES

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The clinical symptoms of haemoglobin disorders such as β-thalassaemia and sickle cell anaemia are significantly ameliorated by the persistent expression of γ-globin after birth. This knowledge has driven the discovery of important regulators that silence γ-globin perinatally. Improved understanding of the γ- to β-globin switching mechanism holds the key to devising targeted therapies for β-haemoglobinopathies. To further investigate this clinically important developmental switch, a novel fluorescent-based cellular reporter assay system was developed. Two fluorescent reporter genes, DsRed and eGFP, were inserted into an intact 183-kb intact human β-globin locus, replacing the coding regions of the $^{G}\gamma$- and β-globin genes, respectively, and was stably transfected into adult murine erythroleukaemic (MEL) cells. Following RNA interference (RNAi)-mediated knockdown of two key transcriptional regulators, Myb and BCL11A, we observed a derepression of γ-globin, measured by DsRed fluorescence and quantitative reverse transcriptase-polymerase chain reaction ($p < 0.001$). Interestingly, double knockdown of Myb and DNA methyltransferase 1 (DNMT1) resulted in a robust induction of ε-globin, (up to 20% of total β-like globin species) compared to single-knockdowns ($p < 0.001$). Conversely, double-knockdowns of BCL11A and DNMT1 enhanced γ-globin expression (up to 90% of total β-like globin species) compared to single-knockdowns ($p < 0.001$). Moreover, following RNAi treatment, expression of human β-like globin genes mirrored the expression levels of their endogenous murine counterparts. These results demonstrate that Myb and BCL11A cooperate with DNMT1 to achieve developmental repression of embryonic and fetal β-like globin genes in the adult erythroid environment.

O9: IDENTIFICATION OF NOVEL INDUCERS OF γ-GLOBIN BY SCREENING EPIGENETIC MODIFIERS USING Gγ-EGFP PRIMARY ERYTHROID CELLS

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β-thalassemia is one of the most common monogenic disorders, caused by mutations within the β-globin gene that lead to insufficient production of β-globin. β-thalassemia patients are treated by monthly blood transfusion. Transfusion therapy is complicated by pathologies of iron overload, necessitating additional therapies. Improved treatments for β-thalassemia are therefore urgently required. Reactivation of the developmentally silenced γ-globin gene is a potential therapeutic strategy for β-thalassemia because the expression of γ-globin compensates for the lack of β-globin. Silencing of γ-globin is an epigenetic process involving chromatin modifications within the β-globin locus, including histone methylation and acetylation and DNA methylation. Inhibiting these processes can reactivate expression of γ-globin. The Gγ-eGFP (GG) transgenic mouse carries the entire human

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β-globin locus, modified to replace the γ-globin genes with eGFP. We have previously demonstrated the use of GG primary fetal erythroid cells to assay compounds for the capacity to reactivate the γ-globin promoter. Flow cytometry detects alterations in eGFP fluorescence, allowing rapid measurement of γ-globin promoter activity in live cells. Using this assay, a library of 90 epigenetic drugs was screened in a 96-well format. Known inducers of γ-globin were identified, including HDAC inhibitors and the inhibitor of DNA methyltransferase decitabine. Several compounds not previously associated with γ-globin gene expression were also identified, targeting various aspects of epigenetic gene regulation. We have therefore demonstrated high-throughput screening for inducers of γ-globin in live primary cells and identified novel compounds for further study using mouse and human adult erythroid cells.

O10: MELLIGEN CELSS: AN INSULIN-SECRETING HUMAN LIVER CELL LINE WHICH IS RESISTANT TO CYTOKINE-INDUCED IMMUNE ATTACK

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Type I diabetes (TID) results from the T-cell mediated autoimmune destruction of the insulin producing pancreatic β cells. Exogenous insulin therapy cannot achieve precise physiologic control of blood glucose concentrations and debilitating complications develop, which greatly increase morbidity and mortality for patients. Gene therapy is one strategy that holds considerable promise to cure TID. As an alternative to the transplantation of islets, a human liver cell line has been genetically engineered to reverse TID. The initial liver cell line (Huh7ins) commenced secretion of insulin in response to glucose at 2.5 mmol/L. After transfection of the Huh7ins cells with human islet glucokinase, the resultant Melligen cells secreted insulin in response to glucose within the physiological range; commencing at 4.25 mmol/L. Melligen cells exhibited increased glucokinase enzymatic activity in response to physiological glucose concentrations, as compared with Huh7ins cells. When transplanted into diabetic immunoincompetent mice, Melligen cells normalized blood glucose within the physiological range of blood glucose concentrations. A quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) revealed that these cell lines expressed a range of ß-cell transcription factors and pancreatic hormones. Exposure of Melligen and Huh7ins cells to pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ) affected neither their viability, nor their ability to secrete insulin to glucose. Microarray and qRT-PCR analyses indicated the survival of Melligen cells in the presence of ß-cell cytotoxins was putatively related to expression of NF-κB and anti-apoptotic genes (such as BIRC3). This study describes the successful generation of an artificial ß–cell line, which, if encapsulated, may offer a clinically applicable cure for T1D.

O11: TEMOZOLOMIDE VERSUS MGMT: TRIALLING A GENE THERAPY STRATEGY TO OVERCOME DRUG RESISTANCE IN THE TREATMENT OF BRAIN TOMOURS

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Methyl-guanine-methyl-transferase (MGMT) is an endogenous DNA repair protein expressed in brain tumour cells which confers resistance to temozolomide. Pharmacological inhibition of MGMT can effectively sensitise tumour cells to chemotherapy but is accompanied by significant toxicity in the bone marrow where endogenous MGMT expression is low. To overcome this limitation, a strategy to render haematopoietic stem cells (HSC) resistant to chemotherapy by gene transfer of an inhibitor insensitive MGMT mutant (MGMTP140K) is being trialled at the Children's Hospital at Westmead. This Phase I safety and feasibility trial uses a 5 day *ex vivo* gene transfer protocol to target G-CSF mobilised, $CD34^+$ selected peripheral blood stem cells (PBSC). It is open to patients with tumours recurring after conventional treatment, or with newly diagnosed brain stem glioma. Six patients have been enrolled, with successful mobilisation of PBSC, gene transfer and re-infusion of gene-modified $CD34^+$ HSC. Post-infusion cycles of chemotherapy aimed at treating the tumour and selecting for gene-modified cells have been given at 3–4-weekly intervals, with the goal of achieving chemotherapy dose escalation. Tumour progression early during the course of post-infusion chemotherapy resulted in three patients being taken off the trial chemotherapy regimen prior to dose escalation. In the remainder, dose escalation of post-infusion chemotherapy was possible, although this was achieved in the absence of chemoprotection by gene modified cells. Gene-modified cells have been detected only at low levels and during a short period following infusion. Improving engraftment of the gene modified cells is the focus of a modification to the conditioning chemotherapy regimen for future patients.

O12: REGENERATING HAIR CELLS IN THE COCHLEA USING ATOH1 GENE THERAPY AFTER HEARING LOSS IN GUINEA PIGS

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An estimated 360 million people suffer from hearing loss worldwide. Sensorineural hearing loss is characterized by permanent loss of cochlear hair cells. Recently, the Atoh1 gene (human homolog of the *Drosophila melanogaster* gene *atonal*) has been investigated as a factor to regenerate hair cells. Encouragingly, Atoh1 gene therapy transforms residual supporting cells into new hair cells; however, many studies use immature animals where hair cell regeneration is more permissive. This study aims to characterize hair cell formation via *Atoh1* gene therapy in profoundly deaf, mature guinea pigs. Guinea pigs were deafened by ototoxic drugs or noise and, 2 weeks later, received adenoviral vectors containing the *Atoh1* gene locally in the left cochlea. After 3–10 weeks, cochleae were assessed for transgene expression and hair cell number, maturity and synaptogenesis. Guinea pig hearing was assessed throughout. There were significantly more hair cells in cochleae over-expressing *Atoh1* compared to the contralateral cochlea and compared to control guinea pigs ($p < 0.05$); however, the number of hair

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cells in *Atoh1*-treated animals was far below normal. The GFP$^+$ *Atoh1* hair cells expressed a number of hair cell markers including myosin VIIa, parvalbumin and calbindin. Expression of the synaptic protein CtBP2 was protected by *Atoh1* expression but not restored to the normal density and location within the cell. There was no evidence of synaptogenesis of auditory neurons with GFP$^+$ *Atoh1* hair cells and hearing was not restored. *Atoh1* gene therapy alone cannot fully convert nonsensory cells into new hair cells after profound or noise-induced hearing loss.

O13: A PHASE 1 GENE THERAPY TRIAL WITH SUBRETINAL RAAV.SFLT-1 FOR THE LONG-TERM TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION: 1-YEAR FOLLOW-UP

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We report the safety and preliminary efficacy of a single subretinal injection of rAAV.sFlt-1to treat wet age-related macular degeneration (wAMD). Eight patients, most of whom received extensive treatment with ranibizumab, were enrolled. Six received rAAV.sFlt-1 and two were part of the control group. During the follow-up period, subjects were treated with ranibizumab according to strict, masked criteria based on visual acuity (VA) and optical coherence tomography (OCT). Laboratory tests included haematology, renal and hepatic function, electrolytes, urine protein and IgM, IgG, IgA and lymphocyte subset analysis. In addition, assays for anti-AAV antibodies, neutralising antibodies and ELISpot were also performed. Ophthalmic safety was assessed by biomicroscopic examination, intraocular pressure (IOP), indirect ophthalmic examination, optical coherence tomography (OCT), colour fundus photography and fluorescein angiography. Neutralizing antibody and clinical laboratory assessments, including blood biochemistry, complete blood count and lymphocyte subsets, generally remained unchanged from baseline. There was no evidence of loss of VA, IOP elevation, retinal detachment, or intraocular or systemic inflammation. OCT demonstrated a decrease or lack of fluid in all subjects. Out of a possible 72 rescue injections for the subjects in the treatment group, two were given. Control subjects received ten times as many rescue treatments during the same period. The average VA in treatment group was 41.8 and 49.3 EDTRS at baseline and at 1 year, respectively. No subjects showed signs of choroidal or retinal atrophy. The results confirm that rAAV.sFlt-1 subretinal injection is safe and well tolerated and a single injection of rAAV.sFlt-1 may provide a durable, long-term treatment option for wAMD.

O14: DEVELOPING NOVEL THERAPIES AND BIOMARKERS FOR FRIEDREICH ATAXIA

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We are using cellular and animal models of the neurodegenerative disease Friedreich ataxia (FRDA) to develop therapies and biomarkers for translation to the clinic. Bone marrow transplant (BMT) and gene therapy are promising treatments for several genetic diseases. Using an FRDA mouse model, we are determining whether BMT provides benefit for FRDA. We examine molecular pathologies and perform behavioural analyses to determine whether there is any functional improvement in the FRDA phenotype. In parallel, we are developing self-inactivating lentiviral vectors that express *FXN*. We will measure *FXN* expression and examine mitochondrial function following gene correction in cells derived from individuals with FRDA. Understanding the relationships between the different aspects of FRDA also enables us to develop novel treatments and biomarkers in a more directed way. In an international collaboration [1], we demonstrated DNA methylation can predict *FXN* expression and clinical outcome, including age of onset and disease severity. We are now building on these data to examine DNA methylation at a genome-wide scale in patient-derived cells aiming to identify the factors involved in the epigenetic modulation of the *FXN* locus. Identifying new FRDA-related genes could also provide insight into disease mechanism and reveal new biomarkers and therapeutic targets.

1. Evans-Galea MV, Carrodus N, Rowley SM, *et al*. *FXN* methylation predicts expression and clinical outcome in Friedreich ataxia. *Ann Neurol* 2012; **71**: 487–497.

O15: AUTOLOGOUS CELL REPLACEMENT FOR HEREDITARY MUSCLE DISEASE

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Autologous regenerative cell replacement is precluded as a viable therapeutic approach in hereditary degenerative muscle disorders such as Duchenne muscular dystrophy (DMD), due to the presence of the disease-causing mutation in all cells from the affected individual. On the other hand, to be functionally effective, cell replacement therapy needs to target at least 20% of the 40% of total body mass representative of muscle tissue in any individual with degenerative muscle disease. Autologous cell replacement therapy for hereditary muscle disease thus requires: (i) accurate corrective gene editing (cGE) strategies to remove the disease-causing mutation from (ii) a relevant myoregenerative cell derived from non-muscle tissue, which needs (iii) effective delivery to maximise corrected loci within the target muscle tissue. Improvement of several cGE technologies combined with developments in induced pluripotent stem cell (iPSC) technology over the past two decades can be integrated with polymer-based cell delivery approaches to make feasible autologous cell replacement therapies for hereditary muscle disease. This presentation communicates the development of a multicomponent cGE/iPSC/polymer system by which to deliver autologous myoregenerative cells to dystrophic muscle in the *mdx* mouse model of DMD. Specifically, this consists of the application of cGE to skin-derived iPSCs from *mdx* mice, their differentiation and expansion *ex vivo* to robust myoregenerative phenotype and delivery to recipient *mdx* muscle within a soft gel Cell/Fibre microtissue construct format to restore functional dystrophin in *mdx* muscle. This forms the basis of an

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autologous regenerative cell therapy for DMD and other hereditary muscle diseases.

O16: SPLICE-MODIFYING ANTISENSE OLIGOMERS: PRECISION MEDICINES TO CORRECT ABERRANT SPLICING

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Antisense oligomer-mediated exon skipping can restore functional dystrophin expression in Duchenne muscular dystrophy (DMD), with consequent clinical benefit in a subset of patients. The most common DMD-causing mutations are frame-shifting deletions of one or more exons, and all clinical studies to date are designed to treat such deletions. We are extending the application of antisense oligomers to less common mutations that impact upon pre-mRNA splicing, including splice site mutations, deep intronic changes activating cryptic exons and small mutations impacting exon selection. Many such mutations are not readily identified during routine diagnostics but may allow expression of near-normal proteins after splice intervention. Such strategies have potential application to selected mutations in almost any gene, and are of particular interest as therapies for rare diseases, of which there are approximately 7000. Eighty percent of rare diseases are genetic in origin and, although each disease has a low incidence, collectively, these conditions present a significant burden in terms of mortality, quality of life and healthcare costs. Treatment options for rare diseases are limited, and implementation of clinical trials for rare diseases is often hindered because of low patient numbers, particularly when the treatments are mutation-specific. Over 25% of all missense and nonsense mutations are predicted to alter pre-mRNA processing and, together with mutations affecting canonical splice sites, are a major cause of human disease. We present strategies to address specific splicing defects, and propose an approach to develop therapeutic compounds for rare inherited diseases that would otherwise be considered beyond the scope of commercial pharmaceutical intervention.

O17: LOCKED NUCLEIC ACID-MODIFIED ANTISENSE OLIGONUCLEOTIDES FOR ENHANCED EXON SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY

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Oligonucleotides have gained significant interest in recent years towards the development of therapeutics and diagnostics against several diseases. The first such drug to enter the clinic has been Vitravene, used for the treatment of cytomegaloviral retinitis. Later, Macugen was approved for the treatment of macular degeneration, and very recently Kynamro against familial hypercholesterolemia. Oligonucleotides composed natural nucleotide monomers pose severe limitations, including poor nuclease resistance and decreased target binding affinity, and are not suitable for therapeutic developments. To overcome these challenges, chemically-modified nucleic acid analogues are used. One of the most prominent and successful analogues is locked nucleic acid (LNA) [1,2]. We are developing LNA and other chemically-modified therapeutic

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oligonucleotides to improve the efficacy and pharmacokinetic properties. Very recently, we have investigated the potential of LNA-modified antisense oligonucleotides (LNA AOs) for exon skipping in Duchenne muscular dystrophy (DMD) [3]. LNA AOs were designed in combination with 2'-OMe-modified RNA nucleotides (20mer and a truncated 18mer LNA/OMe AOs) for targeting exon 23 of the dystrophin gene in mouse (mdx mouse with exon 23 point mutation) primary myoblast cells. A fully-modified 20mer 2'OMe AOs were also used for comparison. The results showed that LNA-modified AOs are highly efficient in exon 23 skipping. Notably, the truncated 18mer LNA/OMe AO also induced efficient skipping of exon 23. These preliminary results clearly indicate that the development of highly potent LNA AOs in combination with various other chemistries could substantially improve the accuracy and efficiency of exon skipping.

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O18: MUSCLE-DIRECTED GENE THERAPIES AS PROSPECTIVE INTERVENTIONS FOR DIABETES AND METABOLIC DISEASE

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Recombinant viral vectors have shown promise as prospective gene therapies for a variety of neuromuscular disorders, and conditions where loss of muscle mass and function occurs. We propose that muscle-directed gene therapies may also have applicability for treating disorders affecting other organ systems. One such example is type 2 diabetes (T2D), which culminates in failure of pancreatic β-cells to produce insulin in response to hyperglycaemia, and the spectrum of complications that ensue with inadequate blood glucose. We have found that interventions comprising recombinant adeno-associated virus-based vectors (rAAV vectors) that manipulate the TGF-β signaling network in skeletal muscle can potently affect the regulation of skeletal muscle mass in mouse models of muscle wasting and frailty. Moreover, we have observed that adaptation of these interventions can markedly ameliorate the development of diabetic pathology in mouse models of T2D. Treatment of diabetic mice prevented hyperglycaemic progression and lowered fasting blood glucose levels, resulting in a correction of haemoglobin glycation and other features of organ function to within the range for nondiabetic mice. Although untreated mice exhibited transient hyperinsulinemia that transitioned to eventual chronic hypoinsulinemia, insulin-producing capacity was maintained in treated mice. Notably, delayed intervention was also found to reverse features of T2D in animals previously exhibiting advanced disease. Existing treatments for T2D largely aim to reduce obesity, increase insulin sensitivity, or augment insulin release. Our data suggest that muscle-directed gene therapy strategies exert beneficial effects in the diabetic setting contributing to the preservation and/or restoration of pancreas function. With a growing body of pre-clinical and clinical data supporting the safety and efficacy of gene therapy approaches targeting skeletal muscle, we propose that further development of muscle-directed gene therapies could prove highly complementary to existing treatment modalities for diabetes and diabetic complications.

O19: THERAPEUTIC ALTERNATIVE SPLICING: AN UPDATE ON DUCHENNE MUSCULAR DYSTROPHY CLINICAL TRIALS AND OTHER APPLICATIONS

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Antisense oligomers (AO) can induce alternative splicing by either blocking positive exon enhancer elements to excise a targeted exon, or masking splice silencer motifs to promote retention of an exon normally excluded from the mature mRNA. We have developed a novel AO-based treatment for Duchenne muscular dystrophy (DMD) by removing exons associated with protein-truncating mutations. Targeting dystrophin exon 51 for excision during pre-mRNA processing will restore the reading frame and functional dystrophin expression in the most common subset of DMD deletion patients. An extended Phase 2 study, initiated by Sarepta Therapeutics in Nationwide Children's Hospital, Columbus, Ohio, has been underway for over 3 years. Treated boys (average age now 13 years) have maintained similar levels of ambulation over the trial period, and their respiratory performance (maximum inspiratory pressure and maximum exhalation pressure) has remained unchanged. No treatment related adverse events have been reported to date. This trial remains ongoing, with additional trials commencing in the UK and the USA. The loss of exon identity, and/or intron definition, is a more common basis for human disease than had been previously appreciated. One study reported that approximately 25% of known pathogenic nonsense or missense mutations caused abnormal pre-mRNA processing, most commonly exon loss. Using DMD exon switching as an exemplar, we are now pursuing therapeutic alternative splicing for other DMD mutations, as well as unrelated disorders. Amenability to splice intervention may be dependent more on the nature of the mutation and consequence on pre-mRNA processing than the gene itself.

O20: GENE DELIVERY TO THE GROWING MURINE LIVER USING A HYBRID RAAV/*PIGGYBAC* SYSTEM RESULTS IN STABLE CORRECTION OF SEVERE METABOLIC LIVER DISEASE PHENOTYPES

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Liver-targeted gene therapy using adeno-associated virus-based vectors (rAAV) shows therapeutic promise in animal models and adult-focused clinical trials. This promise, however, is not directly translatable to the growing liver where rapid clearance of rAAV episomal genomes occurs in concert with hepatocellular proliferation. We have developed a hybrid rAAV/*piggyBac* transposon vector system combining the highly efficient liver-targeting properties of rAAV with stable *piggyBac*-mediated transposition of the transgene into the hepatocyte genome. Transposition efficiency was first tested in newborn wild-type mice following delivery of a rAAV containing

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an EGFP expression cassette flanked by *piggyBac* transposase recognition sequences with or without a second rAAV expressing *piggyBac* transposase. A 20-fold increase in stably gene-modified hepatocytes was observed 4 weeks post-treatment compared to traditional rAAV gene delivery. The therapeutic potential of the system was then modeled in the context of severe early onset UCDs. A single treatment in the perinatal period was sufficient to confer robust and stable phenotype correction in the ornithine transcarbamylase deficient *Spf*^{ash} mouse and neonatal lethal argininosuccinate synthetase knockout mouse. The system was also tested in a murine model of chronic liver disease, progressive familial intrahepatic cholestasis type 3, in which intervention prior to disease onset is necessary due to the development of chronic liver pathology which impedes hepatocyte transduction. Stable persistent expression extending into adulthood was achieved with increased mean biliary phosphatidylcholine concentration and dramatically reduced liver fibrosis. Finally, transposon integration patterns were analysed, revealing 127 286 unique integration sites which conformed to previously published *piggyBac* data.

O21: REPORTER AND THERAPEUTIC GENE TRANSFER TO THE BRAINS OF NORMAL AND MUCOPOLYSACCHARIDOSIS IIIA MICE, RESPECTIVELY, FOLLOWING INTRACEREBROVENTRICULAR DELIVERY OF LENTIVIRUS

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Lysosomal storage disorders are inborn errors of metabolism caused by deficiencies in lysosomal proteins. Approximately two-thirds of cases include central nervous system pathology, which is not amenable to conventional enzyme replacement therapies since lysosomal enzymes do not readily cross the blood–brain barrier (BBB). Emerging gene therapy strategies for these disorders must similarly overcome challenges presented by the BBB. In the present study, lentivirus was administered to adult mice *via* the cerebral lateral ventricles as a means to bypass the BBB and target gene transfer to the brain. Delivery of a reporter vector (LacZ) was initially investigated in normal animals, whereas therapeutic efficacy was assessed using a mouse model of mucopolysaccharidosis type IIIA (MPS IIIA). Following infusion of lentivirus to both lateral ventricles, Lac Z positive cells were observed at the outer surfaces of the cerebral hemispheres, cerebellum and olfactory bulb, as well as along the optic nerve, at the back of the eye, along the spinal cord, and at the choroid plexus. Light microscopic analysis revealed that most observable transduced cells were neurons, usually adjacent surfaces in contact with the CSF, but also in deeper layers of the forebrain. The hippocampus was highly transduced. Transduction of both ciliated and nonciliated ependyma was also observed. For MPS IIIA mice, therapeutic vector was detected in coronal sections spanning the entire brain and, correspondingly, therapeutic enzyme was restored to 0.5- to four-fold of normal levels. The results of this study support the intraventricular injection as a tool to target the brain with therapeutic genes.

O22: TOWARDS CELL THERAPY FOR AN INBORN ERROR OF METABOLISM: TARGETING THE ORNITHINE TRANSCARBAMYLASE LOCUS IN BIPOTENTIAL MURINE OVAL LIVER CELLS USING ADENO-ASSOCIATED VIRUS

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Liver progenitor cells (LPC) are an appealing source of cells for cellular therapy of the liver due to their easy isolation, maintenance of stable phenotype in long-term culture and capacity for differentiation into hepatocytes or cholangiocytes. However, a requirement for autologous therapy of inborn errors of metabolism is repair of mutant loci. Using bipotential murine oval liver cell lines (BMOL) as an LPC model, this study evaluates an adeno-associated virus approach to edit the LPC genome via homologous recombination (AAV-HR) and to determine whether induction of DNA strand-breaks (DSB) using CRISPR technology can augment this process. A targeting vector (OTCe4-neo) was constructed to insert OTC cDNA, polyadenylation signal and a *neomycin phosphotransferase* expression cassette in exon 4 of *Otc*, a design shown to restore OTC expression in *Spf^{ash}* mice. Having confirmed *Otc* vector transduction and targeting by nested polymerase chain reaction (PCR) in a readily available BMOL cell line (TAT-LacZ), BMOL lines were derived from male *Spf^{ash}* mice and validated by expression of LPC markers (E-Cad, A6, EpCAM, CD24 and CK19) using immunohistochemistry. Further characterisation by differentiation into hepatoblasts or ductal epithelial cells when maintained in appropriate *in vitro* conditions is ongoing. By contrast to BMOL-TAT-LacZ, AAV-HR targeting events could not be detected by nested PCR in either of two *Spf^{ash}*-derived lines unless cells were transfected with an *Otc*-specific CRISPR sequence to induce DSB at the target locus. Ongoing studies will determine the relative frequencies of AAV-HR and restoration of OTC expression in this model.

O23: IDURONIDASE TRANSDUCED MSCS IMPROVE THE BEHAVIOURAL DEFICITS IN MUCOPOLYSACCHARIDOSIS I MICE WITH LIMITED BIOCHEMICAL CORRECTIONS

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Mucopolysaccharidoses (MPS) are a group of heritable lysosomal storage disorders, characterized by the loss or reduction of a lysosomal enzyme required for the degradation of glycosaminoglycans chains. Haematopoietic stem cell (HSC) transplant and enzyme replacement therapy correct the soft tissue aspects of MPS disease but have a limited effect on skeletal and neurological symptoms. Mesenchymal stem cell (MSC) therapy has the potential to overcome these limitations due to their ability to differentiate into bone and brain cells. MSCs produce and secrete significantly higher levels of multiple MPS enzymes than HSCs *in vitro* and can be engineered to over-produce MPS enzymes using a lentivirus, suggesting a greater potential for correcting MPS pathology. Lentiviral transduction was stable and persistent *in vitro*, and over-expression of MPS enzyme did not inhibit MSC differentiation down osteogenic, adipogenic, chondrogenic or neurogenic lineages. Systemically administered human MSCs distribute widely and cross the blood–brain barrier. Significantly elevated brain

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iduronidase was observed along with functional improvements in neuromuscular strength, motor control, coordination and spatial learning in the MPS I mouse. However, no correction of lysosomal storage was evident. MSCs were found to limit astroglial activation and modulate the brain expression of *Cd68* and *Tnf*, which could account for the functional changes observed. Overall, MSCs have superior enzyme production and secretion capacity compared to HSCs and can modulate the host environment to limit inflammation, at the same time as promoting functional recovery and repair. However, further work is needed to determine whether behavioural changes were due to MSCs enzyme production or anti-inflammatory properties.

O24: LENTIVIRAL-MEDIATED GENE THERAPY IN THE MUCOPOLYSACCHARIDOSIS TYPE VII MOUSE

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The mucopolysaccharidoes (MPS) are a related group of 11 inherited metabolic disorders that result from a partial or total loss of an enzyme activity required to degrade glycosaminoglycans (GAG) chains. Symptoms common to more than one MPS type include dysostosis multiplex, central nervous system deterioration, airway and cardiac disease, blindness and short stature. When administered within 2 days of birth, lentivirus encoding the missing MPS VII enzyme prevented the build-up of GAG in most somatic tissues, prevented the development of the high bone mass phenotype typical of murine MPS VII, and doubled the lifespan of affected mice. Improvement in behavioural tests was observed, although this was most likely due to a resolution of bone disease rather than a direct effect on neurological function. When administered at 4 months of age, lentivirus encoding MPS VII enzyme rapidly cleared GAG storage in somatic tissues and decreased bone mass to the normal range by increasing osteoclast number and function. In addition, despite achieving normal or supranormal levels of circulating enzyme, treatment did not affect short stature when administered at borth or at 4 months of age. Interestingly, higher transduction efficiency and enzyme levels were achieved in the mild model of MPS VII compared to the severe model of MPS VII and it may be that the higher level of undegraded GAG in the latter model affects lentivirus uptake and thus transduction.

O25: CELLULAR AND GENETIC MEDICINES ADVANCING THE TREATMENT OF METASTATIC PROSTATE CANCER

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Although early stage prostate cancer (PCa) can be cured with surgery, metastatic disease is currently incurable. The prostate is a non-essential organ for life, making gene-directed enzyme prodrug therapy (GDEPT) a feasible option. GDEPT involves the transfer of a non-mammalian prodrug-converting enzyme into the tumour microenvironment. This enzyme renders the prostate tumour sensitive to killing by a systemically administered nontoxic prodrug following

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its conversion to a toxin. GDEPT shows several advantages: prodrug activation in the tumour avoids systemic toxicity; the toxic metabolite readily diffuses across cell membranes killing 'local bystander' PCa cells that do not express the prodrug-converting enzyme; stimulation of anti-PCa immune cells providing PCa metastases killing ('distant bystander effect'). We are currently exploring two GDEPT delivery methods for the treatment of metastatic PCa. The first method utilises the intratumoural injection of a sheep atadenovirus to deliver the bacterial purine nucleoside phosphorylase gene (PNP) under the control of a prostate targeting promoter and in the presence of prodrug fludarabine phosphate (FP253; PCTAU03/00381). FP253 has been registered for a first-in-man Australian Phase I clinical trial (http://ClinicalTrials.gov/NCT00625430). We are now successfully engineering bone marrow-derived mesenchymal stem cells (BMSC) to deliver the yeast cytosine deaminase uracil phosphoribosyltransferase fusion gene (CDUPRT) to metastatic PCa in the presence of prodrug 5-fluorocytosine. BMSC have attracted much attention as cellular gene delivery vehicles due to their ability to seek out cancer anywhere in the body; overcome issues of host immune responses allowing for allogeneic as well as autologous transplantation; and avoid degradation by the immune system, a major limitation of most current gene delivery methods. These novel GDEPT delivery methods show promise for the development of novel therapeutics for PCa patients presenting with metastatic disease.

STUDENT ORAL PRESENTATIONS

OS1: NON-CANONICAL CAPSID ASSEMBLY OF ADENO-ASSOCIATED VIRUS SEROTYPE 5

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With its favorable safety profile and recent therapeutic success, adeno-associated virus (AAV) has great potential as an *in vivo* gene therapy vector. One clinically exciting aspect of this virus is its numerous serotypes, each exhibiting different biological properties. It is important to understand the biological basis for these differences to fully exploit the potential of this vector. AAV serotype 2 has been the most studied serotype to date and recent work on the assembly-activating protein of this serotype (AAP2) has shown insight into the process of AAV2 capsid assembly. AAP2 is a nucleolar-localizing protein that is essential to the capsid assembly process by allowing VP capsid proteins to accumulate in the nucleolus and form capsids. To investigate the properties of AAP for AAV serotype 5 (AAP5), we expressed AAV5-VP3 in HeLa cells in the presence or absence of FLAG-tagged AAP5 by transient plasmid transfection, and looked for localization of AAP5 and AAV5 capsids using anti-FLAG and anti-AAV5 (ADK5a) antibodies. By contrast to AAV2, AAP5 and AAV5 capsids remained largely outside the nucleolus and, surprisingly, ADK5a positive cells could be seen without co-expression of AAP5. To examine the nature of these capsids, we transiently transfected HEK293 cells with the same plasmids and purified the resulting viral capsids by cesium-chloride gradient ultracentrifugation. Electron microscopy revealed particles consistent with the size and shape of intact AAV5 capsids, regardless of the presence of AAP5. So far, our work has indicated that the AAV5-VP3 protein is intrinsically capable of assembling intact capsids.

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OS2: EXPRESSION OF THREE ALLOGENEIC MHC CLASS I IN RECIPIENT LIVER SIGNIFICANTLY PROLONGS SURVIVAL OF FULLY-ALLOGENEIC VASCULARISED CARDIAC ALLOGRAFTS

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In previous studies, AAV-mediated gene transfer of a single mismatched donor MHC class I molecule (Kd or Kb) to C57BL/6 or B10.BR recipient liver, respectively, induced tolerance to skin grafts expressing the same mismatched MHCI molecule. However, such survival is not extended to fully mismatched skin or heart grafts. Tolerance induction may require expression of all mismatched MHC molecules (three class I and two class II). To facilitate expression of multiple MHCI in recipient liver, we created a construct in which the three d-haplotype heavy chains D^d, L^d and K^d were separated by an F2A linker (DaLeK), and then determined the effect of administration of this vector upon heart graft survival. DaLeK was packaged into a liver-specific rAAV2/8 vector. Fully-allogeneic hearts from DBA/2 (H-2^d) were transplanted into C57BL/6 (H-2^b) at either day 7 or day 14 post-inoculation. Administration of 5×10^{11} vector genome copies AAV-DaLeK to C57BL/6 mice yielded strong expression of D^d, L^d and K^d on hepatocytes. Expression was enhanced by co-transduction with a vector encoding β2 microglobulin, ALT levels remained normal and no inflammatory infiltrates were detected. Survival of DBA/2 hearts transplanted into AAV-DaLeK treated mice was prolonged from a MST of 7 days to 23 days. Administration of a control vector did not alter survival. AAV-DaLeK permits the expression of multiple MHCI from a single vector, and its administration significantly prolongs survival of fully-allogeneic heart transplants. A combination of AAV-DaLek with vectors expressing CIITA and/or allogeneic MHC class II may produce tolerance to fully-allogeneic grafts.

OS3: UNDERSTANDING AND HARNESSING MESENCHYMAL STEM CELL HOMING TO METASTATIC PROSTATE CANCER

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Prostate cancer (PCa) is the most frequently diagnosed noncutaneous cancer in Australian men and most PCa-related deaths are associated with metastasis that is currently incurable. Cellular gene therapy utilising bone marrow derived mesenchymal stem cells (BMSC) holds promise as a safe and novel approach for the treatment of PCa. Although our group has identified a unique and safe BMSC subpopulation showing good recruitment to PCa in mice, the distinct receptors and molecular signals required for their homing remain unclear and were explored in this study. GFP tagged BMSC (BMSC-GFP) were infused via the tail vein of syngeneic, immune intact mice in the presence or absence of murine RM1 PCa lung pseudometastases. BMSC-GFP were recruited and persisted within the lungs of RM1-bearing mice alone ($p < 0.01$) compared to nontumour bearing mice. Transwell migration assays were employed to further study BMSC homing to PCa *in vitro*. Over 8 h, BMSCs showed significantly increased migration (two- to three-fold) through a semi-porous (8 μm) membrane in response to RM1 cell conditioned medium (CM), compared to control medium

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(MO) ($p < 0.05$). BMSC migration was abrogated when CM was pre-treated with proteinase K showing a protein mediated response. For identification of genes involved in homing, BMSC were grown in flasks and exposed to CM or MO. After 8 h, cells were harvested for RNA isolation and Affymetrix Mouse Gene 1.0 ST microarray profiling. Partek Genomics Suite software identified 63 up-regulated mRNAs in BMSC exposed to CM. Gene ontology analysis revealed a number of these were involved in chemotaxis. Candidate genes PDGFRα, TGFβR3, CXCL5, CXCL2, CCL2 and CCL7 were validated using TaqMan assays and a real-time quantitative polymerase chain reaction. This study identified several genes involved in BMSC recruitment to PCa that may be exploited for the development of novel cellular gene therapy strategies for metastatic PCa.

OS4: BIOLUMINESCENT IMAGING OF MESENCHYMAL STEM CELL ENGRAFTMENT IN IMMUNE COMPETENT AND IMMUNE DEFICIENT ANIMAL MODELS OF TYPE 1 DIABETES

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The generation of 'artificial' β-cells via gene transfer of pancreatic transcription factors to nonpancreatic tissues as a cell therapy for type 1 diabetes (T1D) has been investigated for over a decade with varying success. Many of the current cell therapies for T1D are limited by the development of immune reactions against the engrafted cells. Consequently, we have suggested the targeting of mesenchymal stem cells (MSCs) for gene therapy due to their reported immune-evasive properties. By modifying bone marrow-derived MSCs (BMSCs) harvested from non-obese diabetic (NOD) mice to express the codon optimised luciferase reporter gene *Luc2*, bioluminescence imaging (BLI) was used to monitor the persistence of engrafted BMSCs in immune competent and immune deficient models of T1D. BMSC-*Luc2* were transplanted subcutaneously in NOD and NOD/*Scid* mice at three cell concentrations (1×10^6, 1×10^5 and 1×10^4 cells/injection) in duplicate, and imaged via BLI following administration of D-luciferin (15mg/ml) for 8 weeks. In NOD mice bioluminescent signal was detected up to 10 days post-transplantation, after which no signal was detected. By comparison, bioluminescent signal was detected in NOD/*Scid* mice up to 8 weeks post-transplantation (experiment ongoing). These data demonstrate that, in immune competent NOD mice, the modified BMSCs are cleared within 2 weeks post-transplantation and suggest immune targeted destruction of the cell graft. Because this model employs syngeneic transplantation of NOD BMSCs, clearing of the cell graft may be due to exogenous expression of the *Luc2* reporter gene. Consequently, the use of luciferase reporter genes for long-term *in vivo* imaging may be limited to immune deficient animal models.

OS5: PREVENTING ATHEROSCLEROSIS WITH A TARGETED GENE THERAPY TO INHIBIT VCAM-1

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The recruitment of leukocytes is an important driver of atherosclerosis development and relies on vascular cell adhesion molecule (VCAM-1) expression on vessel walls. Knockout models and pharmacological interventions show that blocking VCAM-1 can prevent plaque development, yet specific and long-term therapies are still lacking. A fusion construct, CD7/VCAM, has previously been developed which, when expressed in activated endothelial cells *in vitro*, prevents leukocyte transmigration. For long-term *in vivo* delivery of CD7/VCAM, we are now developing a targeted gene therapy system using an adeno-associated virus (AAV) 6 vector. To increase efficacy and safety, a single-chain antibody (scFv) against VCAM-1 is employed to specifically deliver the AAV6 to early stage plaques. Pure scFv has been produced with yields up to 5 mg/L culture, using an insect cell production system. Via flow cytometry, we could demonstrate specific binding to VCAM-1 on SVEC4-10 cells. A variety of site-specific conjugation techniques have been explored to attach the scFv to the AAV6. This approach is based on the combination of chemical glycation, Cu free click chemistry and enzymatic sortase reactions, and has been validated using the covalent attachment of biotin to the AAV6. In the future, we are planning extensive *in vitro* and *in vivo* testing of this targeted gene delivery system, including biodistribution with radiotracers and reporter genes, as well as therapeutic effectiveness. This system represents a one shot, non-invasive and preventative treatment for atherosclerosis. Additionally, it provides a flexible platform technology for similar strategies in other inflammatory diseases and generally for targeted therapeutic gene delivery.

OS6: DEVELOPING BONE MARROW TRANSPLANT AND NOVEL THERAPEUTIC VECTORS TO TREAT FRIEDREICH ATAXIA

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Friedreich ataxia (FRDA) is a debilitating neurodegenerative disorder that decreases the quality of life of affected individuals. FRDA is characterised by numerous clinical symptoms, including progressive ataxia, and patients die young due to cardiomyopathy. Although symptomatic treatments are available to manage disease symptoms and improve quality of life, none have been confirmed to cure FRDA, nor slow the neurodegeneration characteristic of the disease. With limited options in the clinics, the development of new and more effective treatments for FRDA patients to slow disease progression is thus important. Given that the GAA repeat expansion within intron 1 of *FXN* reduces the levels, but does not alter the structure of the frataxin protein, increasing protein levels are anticipated to provide therapeutic benefit to FRDA patients. Our research aims to develop cell and gene therapy, a novel therapeutic application, as a potentially effective treatment for FRDA. We are investigating whether transplant with wild-type bone marrow increases frataxin and improves the phenotype in FRDA mice. Reconstitution of the haematopoietic system with GFP-positive donor bone marrow in corrected recipients indicates successful engraftment following transplant. We have also identified GFP-positive cells in the spinal cord and dorsal root ganglia (DRG) post-transplant, key tissues of pathology. Increased neuronal marking is observed in the DRG and frataxin protein is increased

in several tissues. Neurobehavioural studies are in progress aiming to determine whether transplant can improve the neurological phenotype. These data highlight the corrective potential of BMT to treat FRDA *in vivo* and provide an avenue for the delivery of therapeutic vectors for gene therapy.

OS7: EPIGENETIC THERAPY FOR β-THALASSAEMIA

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Gene silencing by RNA interference (RNAi) is a valuable tool for investigating gene function as well as therapy of genetic disorders. β-thalassaemia is a genetic disorder caused by insufficient production of the β-globin subunit of haemoglobin. Reactivation of developmentally silenced γ-globin can ameliorate the symptoms of β-thalassaemia and is therefore a potential therapy. The mechanism by which γ-globin is developmentally silenced is unclear, alhough epigenetic regulation is known to play a vital role. This research aims to identify novel epigenetic enzymes that regulate γ-globin silencing and subsequently knockdown these genes by RNAi to reactivate γ-globin as a therapy for β-thalassaemia. We screened 914 shRNAs targeted at 130 known and putative epigenetic enzymes using a novel γ-globin-GFP murine erythroleukemic (GG-MEL) reporter cell line. A pooled screening strategy was used: GG-MEL cells were transduced with pooled shRNA viruses, and GFP-positive (representing reactivated γ-globin) 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 silencing. An investigation of the top candidates that emerged from the screen is currently underway. This involves knockdown in murine primary erythroid culture, RNA-seq following knockdown and ChIP-seq. Future experiments will evaluate the therapeutic potential of knockdown of the top candidates in clinically relevant systems, including primary erythroid culture of β-thalassaemia patient cells and β-thalassaemia mouse models.

OS8: EXOSOMAL MICRORNA: POTENTIAL URINARY AND PLASMA BASED BIOMARKERS FOR THE DIAGNOSIS AND PROGNOSIS OF PROSTATE CANCER

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Non-invasive diagnostic tests for prostate cancer (PCa) are urgently needed because measuring serum prostate specific antigen (PSA) has limitations of poor specificity and sensitivity and provides little long-term predictive information. Given the interest in exosomal RNA content in various cancers, we explored the potential utility of exosomal RNA as a basis for a

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diagnostic and prognostic tool for PCa. A panel of candidate exosomal microRNA (exomiR) biomarkers were established by performing an Affymetrix microRNA microarray using RNA extracted from several PCa cell lines (LNCaP, PC3, DU145 and VCaP) and transformed normal prostate epithelia (PNT2). These candidates were validated by a quantitative polymerase chain reaction (qPCR). We then proceeded to gather urine and plasma samples from PCa patients before and following prostate removal surgery, and from healthy volunteers. After extensive optimisations in both body fluids, we can present three methodologies for the extraction of exosomal miRNA from urine and plasma. PCa exosomes are highly enriched with miRNAs. We have identified a panel of exomiRs that differentiate cancerous from normal prostate cells. Importantly, these exomiRs were able to define the androgen dependence status of our cell lines by qPCR analysis. We are currently attempting to translate these findings to the clinic by testing the utility of our unique exomiR panel in the detection of PCa in human urine and plasma. Our preliminary data indicates that our candidate exomiR biomarker panel can differentiate between normal and PCa donor urine/plasma. Exosomal RNAs show great promise as diagnostic/prognostic biomarkers for PCa and will hopefully fill a significant gap in the clinical care of PCa patients.

OS9: MICRODYSTROPHIN AND FOLLISTATIN COMBINATORIAL GENE DELIVERY TO TREAT A SEVERE MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder that results in ambulatory reduction in affected children and premature death from cardiac and/or respiratory failure. DMD is caused by a variety of mutations that result in the loss of dystrophin protein. Gregorevic *et al*. [1] demonstrated that administration of recombinant adeno-associated viral vectors (rAAV6) carrying a truncated dystrophin gene (rAAV6:ΔR4-23/ΔCT, microdystrophin) increased muscle strength and longevity of treated dystrophin$^{-/-}$:utrophin$^{-/-}$ (double knockout: *dko*) mice compared to their untreated littermates. However, wild-type levels of strength were not obtained. To supplement the conferred benefits of microdystrophin, complementary strategies that increase muscle mass and strength were pursued. Follistatin binds and inhibits negative regulators of muscle mass and has been recently confirmed to increase strength after systemic delivery [2]. We hypothesised that co-delivery of rAAV6:FST317 (follistatin) with microdystrophin would increase muscle mass and strength to a greater extent than either gene delivered in isolation. To test our hypothesis, cohorts of *dko* mice received an intravenous injection of follistatin and microdystrophin vectors, alone or in combination, or a control vector. Although mice receiving the combinatorial treatment had an improved muscle mass, we observed deterioration in mice treated with follistatin alone. Subsequently, we investigated the therapeutic benefits attributed to initially stabilising the muscle fibre before inducing muscle growth. We identified that delaying follistatin expression in the duel therapy could increase muscle strength beyond treating with microdystrophin alone. These findings emphasise the potential for a delayed combinatorial gene therapy to ameliorate dystrophic pathology.

1. Gregorevic P, Allen JM, Minami E, *et al*. rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. *Nature Med* 2006; **12**: 787–789.

2. Winbanks CE, Weeks KL, Thomson RE, *et al*. Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. *J Cell Biol* 2012; **197**: 997–1008.

OS10: GENE THERAPY USING RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE 2/8 LIVER-DIRECTED EXPRESSION OF SRAGE IN EXPERIMENTAL TYPE 1 DIABETES

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Type 1 diabetes (T1D) is an autoimmune disease involving the destruction of the insulin-producing pancreatic β-cells. Decreases in circulating concentrations of soluble receptor for advanced glycation end products (sRAGE) confer risk for the development of T1D in children and adolescents. The objective of this study was to deliver sRAGE using recombinant adeno-associated virus serotype 2/8 (rAAV2/8) prediabetes in a T1D mouse model. At day 50 of life, female non-obese diabetic mice ($n = 15$) were intraperitoneally injected with vehicle (4.0% glycerol/PBS), or with rAAV2/8 expressing either enhanced green fluorescent protein (eGFP) or human sRAGE (5×10^{11} vg). T1D incidence was unchanged among groups after 24 weeks ($p = 0.3157$). However, 4 weeks after treatment, macroscopic liver masses were observed in 91% of rAAV2/8-eGFP mice compared to 0% of vehicle and rAAV2/8-sRAGE mice ($p < 0.0001$). Furthermore, oral glucose tolerance tests demonstrated insulin resistance in mice treated with rAAV2/8-eGFP as the insulin to glucose area under the curves ratio was increased compared to mice treated with vehicle (1.4-fold) and rAAV2/8-sRAGE (1.3-fold; $p < 0.01$). Long-term glucose control was improved by rAAV2/8-sRAGE treatment (glycated haemoglobin, 11.15%) relative to its vehicle (14.47%) and rAAV2/8-eGFP (13.75%; $p < 0.01$). rAAV2/8-sRAGE also reduced leukocyte infiltration into the pancreatic islets ($p < 0.05$), a histological hallmark of T1D. These results suggest that rAAV2/8 mediated *in situ* hepatic production of sRAGE has beneficial effects on glucose control and pancreatic leukocyte infiltration, but does not improve T1D incidence. This study also demonstrates that sRAGE reverses certain side effects of rAAV2/8 gene therapy, specifically the formation of liver masses and insulin resistance.

OS11: ANGIOTENSIN-CONVERTING ENZYME 2 GENE THERAPY USING A HIGH EFFICIENCY AND LIVER-SPECIFIC ADENO-ASSOCIATED VIRAL VECTOR ATTENUATES LIVER FIBROSIS IN MICE

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Recent studies suggest that the alternate arm of the renin angiotensin system (RAS) consisting of ACE2 and its peptide product angiotensin-(1–7) [Ang-(1-7)], is a potential therapeutic target in liver fibrosis. We therefore investigated

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the therapeutic effect of ACE2 in mice with experimental liver disease. A single injection of recombinant AAV2/8 carrying murine ACE2 (rAAV2/8-ACE2) with a liver-specific promoter was intraperitoneally administered to mice with liver disease induced by bile duct ligation (BDL) or carbon tetrachloride (CCl_4) injection. The mice were sacrificed 1 week and 6 weeks after treatment in BDL and CCl_4, respectively. Hepatic fibrosis, gene and protein expressions of collagen and pro-fibrotic mediators, and effects on angiotensin II signaling pathways, were analyzed. Untreated mice showed extensive hepatic fibrosis at 2 and 8 weeks after BDL and CCl_4 injections, respectively. However, ACE2 therapy for 1 week (BDL) and 6 weeks (CCl4) significantly reduced fibrosis, as reflected by marked reductions in collagen deposition compared to control vector (rAAV2/8-HSA) injected mice. Moreover, ACE2 therapy significantly downregulated gene expression of collagen-1, α-SMA, CTGF and TGF-β. These changes associated with ACE2 therapy were accompanied by increases in hepatic levels of Ang-(1–7) peptide and reduced Ang II levels, with profound reductions in membrane translocation of the cytoplasmic $p67^{phox}$ NADPH oxidase subunit and activation of p38 and ERK1/2 MAP kinases. We conclude that rAAV2/8-ACE2 reduces fibrosis by changing the intrahepatic balance of Ang II and Ang-(1–7) production in the liver. Thus, ACE therapy may be an effective liver-specific treatment for hepatic fibrosis.

POSTER AND SHORT ORAL PRESENTATIONS

PO1: A NOVEL SYSTEM FOR ANALYSIS AND MANIPULATION OF HUMAN GLOBIN GENE SWITCHING *IN VITRO*

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Over 7% of the global population are carriers of haemoglobinopathies. Despite their prevalence, no effective treatments exist for β-globin disorders beyond lifelong blood transfusions. The globin genes are expressed sequentially during development; thus, different compositions of haemoglobin exist at the embryonic, fetal and adult stages. Individuals with large deletions encompassing the adult β-globin gene exhibit compensatory expression of fetal γ-globin into adulthood, resulting in the benign condition hereditary persistence of fetal haemoglobin. The amelioration of the symptoms of β-globinopathies in the rare cases where these disorders are co-inherited has inspired decades of research into the molecular regulation of haemoglobin switching, with the aim of inducing therapeutic γ-globin expression. Despite this, the mechanism remains unsolved, partly due to the lack of suitable cell and animal models available. Our laboratory has developed a novel system for studying and manipulating haemoglobin switching *in vitro*. We have generated mouse embryonic stem (ES) cell lines harbouring a 200-kB bacterial artificial chromosome (BAC) containing the full human β-globin locus, including its upstream regulatory regions. GFP and DsRed reporter constructs have been inserted under control of the β-globin and γ-globin promoters, respectively (Chan *et al.*, 2012, *The FASEB Journal*). During haematopoietic differentiation of our ES cell lines, we have shown sequential expression of the DsRed and GFP reporter genes by fluorescence microscopy and flow cytometry, confirming that our system recapitulates haemoglobin switching during ontogeny. Using this system, we aim to screen chemical and genetic modifiers of y-globin expression to identify new treatment avenues for β-globinopathies.

PO2: PCR-BASED STRATEGIES FOR DELIMITING LARGE MUTATIONS IN THE DMD GENE

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Large mutations such as deletions, inversions and duplications are causative factors in many genetic diseases, in particular Duchenne muscular dystrophy (DMD). Due to the complexity of pre-mRNA splicing, the genomic span of such mutations is not readily predictable from the sequence of their mature mRNAs, nor vice versa. The difficulty of delimiting these mutations is compounded further in large genes with substantial intronic complements, such as the human dystrophin gene *DMD,* and the cost of whole genome sequencing, although continuing to fall, remains prohibitive for routine diagnosis and researchers attempting to define multiple large mutations. We outline several polymerase chain reaction-based strategies for defining the precise junctions of large gene rearrangements. These techniques represent a practical alternative to whole genome sequencing for defining such mutations to a single gene. We report a pilot study designed to verify the feasibility of these techniques on a subset of patients with large mutations to the *DMD* gene. This study also analyses the mRNAs of these patients and attempts to draw inferences about the effect these mutations have on pre-mRNA processing. The data gleaned from this project and similar future studies will contribute towards our understanding of pre-mRNA splicing, the spliceosome and motifs that impact on gene expression. In a proportion of DMD cases, the phenotype and genotype do not correlate, and elucidating the mechanisms responsible will allow more accurate diagnosis and inform personalized treatment strategies.

PO3: OPTIMISING TARGETED GENOME EDITING OF THE HUMAN β-GLOBIN LOCUS USING CRISPR/CAS9

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β-Thalassaemia is an inherited blood disorder caused by insufficient β-globin synthesis. Many mutations at the β-globin locus responsible for the disease phenotype have been identified. Gene correction through targeted genome editing would be the optimal treatment for patients with β-thalassaemia and other genetic disorders. The recent adaptation of the CRISPR/Cas9 nuclease system for site-directed genome editing in mammalian cells provides an ideal tool for this therapeutic approach. However, precise gene correction relies on the activity of the cellular homology-directed repair (HDR) pathway, which is overshadowed by the nonhomologous end joining (NHEJ) pathway, a process associated with disruptive nucleotide deletions and insertions ('indels'). To identify interventions that bias genome editing towards HDR using CRISPR/Cas9, we developed an assay that simultaneously detects HDR and NHEJ frequencies by targeting only a single nucleotide in the fluorescent reporter GFP. Successful nucleotide substitution via HDR converts GFP to BFP, resulting in a shift in the fluorescence excitation and emission spectra that can be detected by flow cytometry. Conversely, nucleotide indels consequent to NHEJ destroy the function of the reporter, detected as a loss of GFP fluorescence. In both K562 and in HEK293T cells expressing GFP, we demonstrate targeted modification of GFP. HDR occurred with a frequency of 7.8% in HEK293T cells and 2.1% in K562

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cells, corresponding to approximately 10% of the frequency of NHEJ in both cell lines. This assay will be an excellent starting point from which compounds enhancing HDR can be identified in a high-throughput screen. We aim to develop an optimised protocol for HDR for use in hematopoietic stem cells.

PO4: HARNESSING INTRONIC DELIVERY OF SHRNAS FOR HAEMOGLOBIN DISORDERS

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RNA interference (RNAi) has attracted considerable attention because of its great potential in therapeutic applications. Despite the early excitement of gene-specific silencing, significant hurdles involving tissue-specific delivery have limited its clinical application. Here, we demonstrate the therapeutic potential of RNAi by combining the utility of human β-globin gene expression with the intronic delivery of shRNAs. Using a lentiviral vector (LV) expressing the human β-globin gene, we targeted two different genes in murine erythroleukemia (MEL) cells. We first targeted GFP expression in MEL cells that constitutively express GFP. Following transduction of the LV β-globin vector containing GFP-specific shRNA, we demonstrated a significant reduction of GFP expression that positively correlated with human β-globin gene expression and erythroid differentiation. We next targeted α-globin expression in MEL cells. shRNAs targeting either exon 3 or the 3' UTR reduced α-globin mRNA levels by approximately 20% and 40%, respectively. To further validate the utility of this approach in a clinically relevant model system, we transduced primary fetal liver cell derived from the β-thalassaemia $Hbb^{th3/th3}$ mouse. Transduction of the LV β-globin gene therapy vector containing α-globin-specific shRNA reduced murine α-globin mRNA by approximately 60% in primary erythroid cells. This approach is anticipated to synergise with β-globin gene expression to normalise the ratio of α:β-globin chains, thereby avoiding the excess α-globin toxicity responsible for anaemia in β-thalassaemia. Our results demonstrate that the β-globin gene can be harnessed to deliver shRNAs to inhibit target gene expression in erythroid cells and could potentially further improve human gene therapy for haemoglobin disorders.

PO5: EVALUATING THE RELATIONSHIP BETWEEN THE DISTRIBUTION, TRANSFECTION EFFICIENCY AND IMMUNOLOGICAL RESPONSE OF PEGYLATED AND NON-PEGYLATED CATIONIC LIPOPEPTIDE DNA COMPLEXES

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The advancement of intramuscular nonviral DNA vaccines has been hindered due to its poor immunological responses *in vivo*, attributed to its low transfection efficiency. Cationic DNA complexes are considered to interact with extracellular proteins upon injection in the muscle,

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leading to the restricted movement of the complexes *in vivo* and, thus, result in a low number of cells transfected. In our study, we set out to explore the effect of the addition of a poly(ethylene glycol) (PEG) coating to these complexes to shield the positive surface charge, in the hopes of increasing the complexes' distribution *in vivo*. To do so, we developed two novel DNA complexes to test: (1) a cationic lipopeptide/DNA complex and (2) a PEGylated lipopeptide/DNA complex. Results of DNA-based quantitative polymerase chain reactions indicated that similar amounts of the complexes were retained in the muscle after 24 h. However, luciferase reporter assays indicated that the PEGylated complexes yielded overall higher transgene expression than the cationic complexes, with a 200-fold higher activity than the cationic lipopeptide treated mice at 1 month post-injection ($n = 4$; $p < 0.0001$). Antibody response assays showed that the PEGylated complexes elicited a slightly higher titre than the lipopeptide complexes; however, the results were not statistically significant. An *in vivo* ovalbumin-induced cytotoxic T lymphocyte elimination assay, however, showed that both the PEGylated complexes and naked DNA stimulated a significant cellular response, with both eliminating a higher number of target cells (26.7% and 22.5%, respectively) ($n = 8$, $p < 0.001$) than the cationic lipopeptide treated mice (14.3%).

PO6: CAN EXPRESSION OF ALLOGENEIC MHC CLASS II IN RECIPIENT LIVER INDUCE REGULATORY TRANSPLANTATION TOLERANCE?

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Regulatory tolerance to allogeneic cardiac grafts after donor MHCII gene transfer to recipient bone marrow is reported. Allogeneic MHCII is strongly expressed in recipient liver following adeno-associated virus (AAV)-mediated gene transfer, accompanied by a dose-dependent increase in liver T regs. However, survival of allogeneic heart grafts in transduced recipients is unchanged. Hepatocytes are not professional APC, lacking significant expression of co-stimulatory molecules, and chaperones required for antigen processing and presentation. To determine whether augmenting expression of molecular chaperones and/or co-stimulatory molecules by hepatocytes would facilitate induction of allograft tolerance. C57BL/6 mice received 1×10^{11} vgc AAV2/8 encoding class II transactivator (CIITA) and 5×10^{11} vgc IA^d or IA^d alone. MHCII, co-stimulatory molecules, chaperones and inflammatory infiltrate were assessed. IA^d-binding peptides eluted from livers expressing IA^d alone or IA^d/CIITA, were identified by mass spectrometry. DBA/2 hearts were transplanted at day 7 or day 30 post-inoculation. CIITA transduction upregulated expression of native IA^b. Expression of H-2M α and β and of Invariant chain were increased 70- to 500-fold by CIITA, attaining levels comparable to those in spleen. Peptides eluted in the presence of CIITA conformed to the IA^d-binding motif. Expression of co-stimulatory molecules on hepatocytes was not increased by CIITA. Survival of DBA/2 grafts was not altered by the addition of CIITA to IA^d. Expression of CIITA and a single mismatched MHCII in hepatocytes was not sufficient to confer tolerance to fully-allogeneic heart grafts. Ongoing experiments are evaluating the combination of two mismatched MHCII and/or CD86 with CIITA.

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PO7: A SPANNER IN THE WORKS OF HUMAN TRANSFORMER 2 β1 AUTOREGULATION MAY REDUCE THE SEVERITY OF SPINAL MUSCULAR ATROPHY

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Modifier genes involved in pre-mRNA splicing have potential as novel therapeutic targets for reducing the severity of genetic diseases. Spinal muscular atrophy (SMA), is a devastating neurodegenerative disease caused by homozygous loss of the survival motor neuron 1 (*SMN1*) gene. The absence of SMN is embryonic lethal but humans have one or more near-identical copies of *SMN2* that provide low levels of full length *SMN* (FL-*SMN*). A single nucleotide change in *SMN2* exon 7 creates a splice-silencer and leads to the predominant production of Δ7-*SMN*. Human transformer protein 2 beta 1 (TRA2β1), binds a splice-enhancer element in *SMN* exon 7, contributing to the production of FL-*SMN*. Studies show that increasing TRA2β1 concentration *in vitro* leads to 80% of the *SMN2* transcripts being FL. We upregulated TRA2β1 by interrupting its autoregulation mechanism with antisense oligonucleotides (AOs). When TRA2B1 is in molar excess it binds to splice-enhancer motifs in exon 2 of the *TRA2β* pre-mRNA, causing an increase in nonfunctional alternative transcripts containing exon 2 and a concurrent decrease in the *TRA2β1* transcript. We transfected SMA patient fibroblasts with 2'O-methyl AOs targeting exon 2 of *TRA2β* to interfere with TRA2β1 autoregulation. Fibroblasts were harvested at various time points for transcript analysis. Quantitative polymerase chain reaction (PCR) of the *TRA2β* transcripts showed a decrease in the alternative transcripts and an increase in *TRA2β1* transcripts. Reverse transcriptase-PCR showed an corresponding increase in FL-*SMN*. TRA2β1 corrects the splicing of *SMN2* to produce more FL-*SMN*; therefore, up-regulating it may be a useful complementary approach in developing a therapy for SMA.

PO8: THE GENERATION OF A TRANSPOSON-MEDIATED SYSTEM FOR DIRECT TRANSCRIPTIONAL REPROGRAMMING TO NEPHRON PROGENITORS

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Cellular reprogramming holds great promise for the development of desperately needed novel treatment options for chronic kidney disease (CKD). The functional units of the kidney, known as nephrons, arise from a population of embryonic nephron progenitors (NPs). Near birth, a final wave of nephrogenesis depletes this population, rendering the mature kidney unable to form new nephrons regardless of damage or disease. Recreation of NPs may allow regeneration of entire nephrons, making the NP population an ideal target cell for cellular reprogramming approaches to generate alternate CKD treatment options. Using a lentivirus-mediated screen, we identified six transcription factors (*SIX1, SIX2, HOXA11*, *OSR1, EYA1* and *SNAI2)* sufficient to re-impose a NP-like state when co-expressed in adult human kidney epithelial (HK2) cells (Hendry *et al*., *JASN* 2013). To improve this reprogramming and allow

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transferability to *in vivo* models of kidney disease, we have now developed a multicistronic transposon construct. The reprogramming transposon was generated by engineering all six genes into a *piggyBac* construct with intervening 2A sequences, a tetracycline response element for doxycycline inducibility and a reporter (mCherry) for cell enrichment. Co-transfection of HK2 cells with this transposon, a tetracycline activator transposon and a *piggBac* transposase construct, results in tightly regulated doxycycline-inducible mCherry expression and re-expression of NP markers. Delivery of such cells into a neonatal mouse kidney results in contribution to nephron formation. These results demonstrate the feasibility of transposon-based reprogramming to NPs, bringing us closer to realizing patient-specific reprogramming to NPs for cellular therapies, bioengineering applications and nephrotoxicity screening.

PO9: ANTISENSE OLIGONUCLEOTIDES INDUCED SPLICE SWITCHING OF FN1 TRANSCRIPT

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Fibronectin, encoded by *FN* gene, is a glycosylated protein found on cell surfaces, in extracellular matrix and plasma. It is involved in many events, including cell adhesion, migration, wound healing and blood coagulation. Alternative splicing of the *FN1* transcript produces up to 20 transcript variants, which are tissue and developmentally dependent and also regulated by physiological and pathological conditions. It has been shown that the presence of a fibronectin isoform containing an extra domain A (EDA^+-FN), normally absent from the circulation, may be an indicator of chronic inflammation and also promotes ischemia/reperfusion brain injury through a TLR4-dependent mechanism. We reduced the levels of the EDA^+-FN isoform using splice switching antisense oligonucleotides (AO). AOs, comprising 2'-O-methyl modified bases on a phosphorothioate backbone, were designed to exclude the EDA domain in exon 33 from the *FN1* transcript. The ratio of EDA^+-FN to EDA^--FN spliceoforms was analysed 24 h after the fibroblasts were transfected with AOs as cationic lipoplexes. The most efficient AO targeting exon 33 was able to induce an approximately 70% reduction in EDA^+-FN transcript in treated cells. Splice switching AOs are emerging as powerful tools in therapeutic alternative splicing. The exon skipping AO identified in this study will be further investigated for therapeutic potential in pathological conditions caused by elevated level of the EDA^+-FN isoform.

PO10: COMPARISON OF THREE DIFFERENT MUTATIONS IN DMD EXON 40 THAT INFLUENCE THE EFFICIENCY OF AO INDUCED EXON SKIPPING

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Duchenne muscular dystrophy (DMD) is a recessive X-linked disorder affecting one in 3500 boys. The disease is caused by mutations in the dystrophin gene (*DMD*) that result in protein truncation and ablate functional protein expression. Becker muscular dystrophy (BMD), a milder allelic form of DMD is less severe, less common and the progression is much slower, sometimes manifesting later in life. BMD is

caused by mutations in the dystrophin gene that lead to reduced production of the dystrophin protein, which may be of variable quality, therefore increasing the variation of symptoms. Although there is currently no cure for DMD, antisense oligonucleotide (AO) induced exon skipping to restore the reading frame has shown promise. Clinical trials to re-frame the transcript around the most common DMD-causing genomic deletion are underway. Small intra-exonic insertion/deletions and nonsense mutations account for approximately 25% of *DMD* mutations and, although single in-frame skipping of an exon should generally result in a highly functional dystrophin isoform, there is the possibility of the mutation influencing pre-mRNA processing. Here, we report three different mutations in *DMD* exon 40 that influence the efficiency of AO induced exon skipping. Each of the patients in this study would benefit from re-framing of the DMD transcript by skipping of exon 40, although the AO therapy may need to be delivered on an individualized basis due to mutation specific effects on splicing. The influence of the different mutation exon 40 skipping was less pronounced when oligomers were synthesized as a phosphorodiamidate morpholino (PMO) than when 2'O methyl phosphorothioate AOs were used. The PMO demonstrated greater specificity and splice switching capability and may reduce the mutation-specific influences on splicing.

POSTER PRESENTATIONS

P11: CHROMATIN-MODIFYING DRUGS ALTER γ-RETROVIRAL VECTOR INTEGRATION SITE DISTRIBUTIONS IN HUMAN CD34$^+$ HAEMATOPOIETIC STEM/PROGENITOR CELLS

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Previous studies have implicated γ-retroviral (γ-RV) vectors in insertional oncogenesis owing to their propensity to integrate near transcription start sites (TSSs) of proto-oncogenes in haematopoietic cells. This project aims to evaluate the use of two therapeutically recognised chromatin-modifying agents during transduction with integrating vectors to change the pattern of integration in human CD34$^+$ haematopoietic stem/progenitor cells. Human CD34$^+$ cells were isolated from mobilised human peripheral blood. Cells underwent pre-stimulation in a cytokine cocktail prior to transduction, with the addition of 5-Aza-2′-deoxycytidine (10^{-6} M) for the last 16 h. Cells subsequently underwent three rounds of transduction with a γ-RV vector encoding methyl-guanine-methyl-transferase (MGMT) (cumulative multiplicity of infection of 4.5), with addition of trichostatin A (5 ng/mL) during transduction (n = 3). CD34-positivity and MGMT expression were measured after transduction via fluorescence-activated cell sorting. To ensure that the use of these drugs does not impact on the multilineage reconstitution potential of the cells, busulphan-conditioned NSG mice were engrafted with cells transduced with/without drugs (n = 4). Mice were kept for 26 weeks, with all treatment groups exhibiting complete lymphocyte reconstitution without adverse side effects. Genomic DNA junction fragment libraries were constructed from three biological replicates of cells subjected to control and drug conditions and sequenced via paired-end sequencing using

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Illumina MiSeq chemistry. Preliminary data show that addition of drugs reduces TSS-proximal integration compared to control cells, providing evidence that clinically relevant drugs can be used to alter the pattern of integration in γ-RV-transduced cells without compromising engraftment potential. Ongoing work applied to lentiviral integration is aimed at further exploring this paradigm.

P12: GENE THERAPY FOR X-LINKED AGAMMAGLOBULINAEMIA: DEVELOPING LENTIVIRAL VECTORS TO TARGET THE B CELL COMPARTMENT

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X-linked agammaglobulinaemia (XLA), the most common primary immunodeficiency in man, is characterised by an absence of B cells and antibody production due to mutations in the *BTK* gene. Current treatments are noncurative and require lifelong immunoglobulin replacement therapy with protracted morbidity and reduced quality of life. As the prototypic B lymphocyte disorder, XLA is a compelling target for gene therapy; unlike disorders of T cell development, treatment may be extended to both paediatric and adult populations because B lymphocyte ontogeny does not involve the thymus which undergoes age-dependent involution. Given the major disease manifestations observed in patients with XLA is the result of a block in B cell differentiation, previous studies investigating the utility of gene therapy for XLA have considered the use of B cell lineage-restricted promoters to drive Btk expression and avoid the potentially deleterious effects of ectopic protein expression. Our current work investigates the relative merits of using either ubiquitous or B cell lineage-specific promoters in the context of a lentiviral vector system for XLA gene therapy. Using an in-fusion assembly strategy, we have generated a library of lentiviral vectors comprising different enhancer and promoter fragments and used this to transduce the human Namalwa B cell line. Gene-marked cells were obtained by fluorescence-activated cell sorting and Sanger sequencing used to identify a total of 25 unique enhancer/promoter combinations from polymerase chain reaction amplicons. Selected vectors were used to transduce the human HSC-93 and Namalwa B cell lines where we observed between 34- and 45-fold differences, respectively, in the intensity of EGFP expression. We are currently evaluating the utility of candidate vectors for XLA gene therapy by engrafting immunodeficient NSG mice with gene-modified human $CD34^+$ cells.

P13: ADENO-ASSOCIATED VIRUS VECTORS ENCODING SHRNA SPECIFIC FOR ANTI-4-HYDROXYPHENYLPYRUVATE DEHYDROXYGENASE INDUCE ATAXIA AFTER NEONATAL INJECTION

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Persistence of episomal adeno-associated virus (AAV) provirus is transient in dividing

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hepatocytes and homologous recombination mediated by the vector occurs at frequencies too low to produce therapeutic benefit for many diseases with currently available capsids. This project seeks to develop a pharmocoenetic strategy to positively select gene-modified hepatocytes *in vivo* via modulation of the tyrosine catabolic pathway (Paulk *et al*., *Molecular Therapy* 2012), which is active in liver and kidneys. A key requirement of the approach is to genetically reduce expression of an enzyme(s) proximal to fumarylacetoacetate hydrolase, such as 4-hydroxyphenylpyruvate dehydroxygenase (HPD). An *in vitro* assay of six anti-HPD shRNA identified two with the most potent activity in reducing murine HPD mRNA levels, which were subcloned into AAV constructs under the transcriptional control of H1 or U6 promoters. Unexpectedly, shRNA vectors packaged in capsid serotype 8 induced ataxia after intra-peritoneal injection of neonatal mice (2.75×10^{11} vg per pup). Mice exhibited difficulty ambulating at weaning, dragged their hind limbs and, in severe cases, were paralysed and culled for ethical reasons. Ataxia was not observed after similar delivery of AAV encoding a scrambled control shRNA or when two-fold more vector was given to adult mice. We currently hypothesise that vector knockdown of HPD induces neurological impairment in neonatal mice. Further experimentation will determine whether: (i) HPD is expressed in neurons; (ii) AAV vectors delivered to neonates enter the central nervous system; and (iii) HPD-specific shRNA reduces HPD expression in the central nervous system.

P14: REACTIVATION OF γ-GLOBIN USING CRISPR-CAS9 AS A POTENTIAL GENE THERAPY FOR β-THALASSEMIA

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β-thalassemia and sickle cell anaemia are single-gene disorders caused by mutations in the β-globin gene. In cases where β-thalassemia is co-inherited with the condition hereditary persistence of fetal haemoglobin (HPFH), fetal γ-globin production is increased and is able to compensate for loss of adult β-globin. Because of this, understanding the regulation of human blood development and, more specifically, reactivation of developmentally silenced fetal γ-globin has been a research area of much focus. To investigate molecular methods for reactivation of foetal haemoglobin, our laboratory has generated a novel system that recapitulates human blood development *in vitro*. This system utilises mouse embryonic stem (ES) cell lines carrying a 200-kb bacterial artificial chromosome (BAC) encompassing the whole human β-globin locus. A GFP reporter has been inserted under control of the γ-globin promoter, and a DsRed reporter under the control of the β-globin promoter (Chan *et al*., *The FASEB Journal* 2012). This system therefore allows for easy read out of globin expression during erythroid differentiation of ES cells, via fluorescence microscopy and flow cytometry. Using this system, we aim to screen genetic modifiers of globin expression using the CRISPR-Cas9 system to induce HPFH mutations in the proximal γ-globin promoter, with the ultimate aim of discovering new gene therapy approaches for treatment of haemoglobinopathies such as β-thalassemia.

P15: USE OF VIRAL VECTORS IN PRIMARY CELLS

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Viral vectors are appealing vehicles to deliver genes or shRNAs because they are capable of transducing many nondividing or quiescent cell types. This is important for primary cells, which are typically refractory to transfection. Transduction efficiency can vary widely between cell types and it is often prudent to trial several different vectors to achieve the best outcome. Here, we used a panel of lentiviral and adeno-associated virus (AAV) vectors to transduce two primary cell types: human T-cells and mouse cumulus cells. Both cell types are currently used for studies of human disease and efficient gene transfer is needed for disease modelling. Six AAV serotypes (AAV-1, AAV-2, AAV-5, AAV-8, AAV-9 and AAV-DJ) and one VSV-G-pseudotyped lentivirus were used to transduce mouse cumulus-oocyte complexes with transduction efficiency assessed using fluorescence microscopy. Within AAV serotypes, serotype DJ produced the highest transduction levels with AAV-2 and AAV-8 also transducing cumulus cells. Lentivirus transduction was also successful with up to 50% of cumulus cells showing fluorescent protein expression. No oocyte transduction was observed with AAV or lentivirus. We also used lentivirus to transduce primary human regulatory T cells. Two titre-matched lentiviruses expressing GFP under the control of two different promoters were used for transduction. When assessed by fluorescence microscopy and fluorescence-activated cell sorting, GFP expression driven by the human EF1a promoter was stronger than that driven by the cytomegalovirus promoter, despite the latter having performed extremely well in other cell types. These results demonstrate the usefulness of viral vector panels to optimise gene transfer in primary cells.

P16: TRANSGENE EXPRESSION IN GENE-MODIFIED CELLS FOLLOWING *PIGGYBAC*-MEDIATED TRANSPOSITION IS MORE EFFICIENT THAN FROM STABLY MAINTAINED ADENO-ASSOCIATED VIRAL VECTOR PROVIRUS

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Our group has developed a hybrid recombinant adeno-associated virus (rAAV)/transposon system based on stable *piggyBac*-mediated transposition to improve the persistence of transgene expression following rAAV gene delivery to the growing liver. Prototypic rAAV genomes exist predominately as episomes, which are lost in concert with liver growth as a result of hepatocellular proliferation and accompanied by a reduction in therapeutic efficacy. This remains the major translational challenge to paediatric gene therapy for metabolic/genetic liver disorders. Using the hybrid rAAV/transposon system, therapeutic levels of transgene expression were maintained until adulthood in two mouse models with deficiencies in either ornithine transcarbamylase (OTC) or argininosuccinate synthetase following a single intraperitoneal injection either neonatally or *in utero*, respectively. Interestingly, in addition to unprecedented levels of stable gene transfer, we observed significantly higher levels of transgene expression from transposed expression cassettes than from stably maintained rAAV provirus. In wild-type mice, using an EGFP reporter gene, the level of protein expression following co-delivery of the rAAV/transposon and *piggyBac* transposase was 43-fold greater

than in the absence of *piggyBac* transposition, whereas vector copy number was increased only seven-fold. Similar results were obtained in OTC-deficient mice following delivery of the OTC transgene (126-fold increase in OTC activity with only a three-fold increase in vector copy number). Provirus integration is reportedly accompanied by vector genome re-arrangement, deletions and insertions, which may all have negative effects on transgene expression. The hybrid rAAV/transposon system is therefore not only capable of achieving persistent levels of therapeutic transgene expression, although this is possible with a lower effective dose.

P17: COMPARISON OF VARIOUS TRANSFECTION METHODS IN HUMAN AND BOVINE CULTURED CELLS

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Transfection is a gene delivery tool that is a popular means of manipulating cellular properties, such as induced pluripotent stem cell generation by reprogramming factors (Yamanaka factors). However, the efficiency of transfection needs to be improved. In the present study, three transfection protocols [non-liposomal transfection (NLT), magnetofection and electroporation] were compared by analysis of their transfection efficiencies and cell viabilities using human dental pulp cells (hDPC) and bovine fetal fibroblasts (bFF) as cell sources. Enhanced green fluorescent protein gene was used as the delivery indicator. For magnetofection, Polymag reagent was administrated. NLT, FuGENE-HD and X-treme GENE 9 DNA transfection reagents were used for NLT. For electroporation, the Neon™ and NEPA21™ electroporators were tested. Neon™ electroporation showed highest transfection efficiency compared to NLT, magnetofection and NEPA21™ electroporation, with a transfection efficiency of approximately 33% in hDPC and 50% in bFF, based on viable cell population in each cell type. These results suggest that transfection by Neon™ electroporation can be used to deliver foreign genes efficiently in human and bovine somatic cells. Our study was supported by a grant from the National Research Foundation of Korea (No. 2014050477 through the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University).

P18: FUNCTIONAL VALIDATION OF LENTIVIRAL VECTORS FOR X-LINKED AGAMMAGLOBULINAEMIA CAN BE EVALUATED BY A FLOW CYTOMETRY CALCIUM FLUX ASSAY

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X-linked agammaglobulinaemia (XLA) is an immune deficiency caused by a mutation in the gene encoding Bruton's tyrosine kinase (Btk). XLA patients do not have mature B cells and immunoglobulin and present with recurrent bacterial infections requiring lifelong immunoglobulin treatment. Gene therapy offers the option of 'cure' for this condition. Btk is required for pre-B-cell expansion and B-cell receptor (BCR) signalling. A downstream result of BCR signalling is calcium flux. Calcium (Ca^{2+}) plays an important role in B cell activation and its signals control proliferation, differentiation, apoptosis and multiple Ca^{2+}-sensitive transcription regulators. The aim was to evaluate (i) the best method to perform

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a Ca^{2+} flux assay by flow cytometry comparing fluorescent Ca^{2+} indicators excited with visible light versus those excited by ultraviolet (UV) light and (ii) to determine the sensitivity of the assay by assessing the number of transduced cells (i.e. functioning cells) that are required to achieve a positive flux. A lentiviral vector encoding Btk was designed and assessed in a Btk-deficient DT40 (chicken lymphoma) cell line. We found that a Ca^{2+} flux assay by flow cytometry using UV excitation was easier to perform, analyse and gave clearer results. A dilution experiment using a transduced DT40 Btk positive clone mixed with untransduced cells was undertaken to determine the level of sensitivity of the assay. Functional assessment of viral vectors *in vitro* using a Ca^{2+} flux assay prior to *in vivo* experiments is a valuable tool in working towards gene therapy for XLA.

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