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8th AUSTRALASIAN GENE THERAPY SOCIETY MEETING

Date

May 8 – May 10, 2013

Venue

Aerial UTS Function Centre
University of Technology, Sydney
Ultimo, NSW, Australia

The Australasian Gene Therapy Society (AGTS) held its Seventh Biennial Meeting between 8th May and 10th May 2013. The AGTS 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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KEYNOTE PRESENTATIONS

KEYNOTE 1: RETROVIRAL DNA INTEGRATION: MECHANISMS AND APPLICATIONS TO HUMAN GENE THERAPY

Sponsored by the Children's Medical Research Institute

Frederic D. Bushman[1] Troy Brady[1], Frances Male[1], Nirav Malani[1], Philippe Leboulch [4], Salima Hacein-Bey, Marina Cavazzana-Calvo[3], and Charles C. Berry[2], [1]University of Pennsylvania School of Medicine, Department of Microbiology, 3610 Hamilton Walk, Philadelphia, PA 19104-6076, [2] Department of Family/Preventive Medicine, University of California, San Diego School of Medicine, San Diego, CA 92093, [3]Department of Biotherapy, Hôpital Necker-Enfants Malades, 75743 Paris, France, [4]CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay-aux-Roses 92265, France

Integration of therapeutic sequences is widely used to achieve stable gene correction during human gene therapy. The lecture will cover the mechanisms underlying retroviral DNA integration and the consequences for the host cell, using examples from ongoing gene therapy trials. Specific topics to be discussed will be HIV integration in cells, targeting via tethering by LEDGF/p75 protein, and outcome of recent gene therapy trials to treat SCID-X1, beta-thalassemia, and other diseases.

KEYNOTE 2: rAAV6-DESMIN PRESERVES HEART FUNCTION IN A MOUSE MODEL OF DESMINOPATHY

Darren Bisset[1], Galina Flint[2], Rachel M. Faber[1], Jeffrey S. Chamberlain[1], Michael Regnier[2], and Glen B. Banks[1], [1]Department of Neurology, [2]Department of Bioengineering, University of Washington, Seattle, WA, 98115, USA

Desmin is the primary intermediate filament protein of striated muscle that connects the sarcomeres to the sarcolemma and organelles. Mutations in desmin generally cause a dominant-negative affect on filament formation leading to a severe dilated cardiomyopathy, skeletal muscle weakness and death. It is likely that a prospective gene therapy approach will require both knocking down the mutated allele and replacing the mutated allele with wild-type desmin. To demonstrate a proof of principle that desmin can be functionally replaced we treated desmin knockout mice with 8e12 vector genomes of rAAV6 packaging a codon-optimized human desmin under the control of the muscle-specific MHCK7 promoter. The mice were treated systemically at 2 weeks of age and examined at 1 year of age by echocardiography. The desmin knockout hearts developed a severe dilated cardiomyopathy. The left ventricle was significantly dilated during systole and diastole when compared to wild-type mice. The desmin knockouts also had significantly reduced fractional shortening and ejection fraction when compared to wild-type controls. The left ventricle inner diameter in treated mice was maintained at wild-type levels. The fractional shortening and ejection fraction were also significantly improved in the treated mice, beyond wild-type controls. We are currently measuring the hemodynamics and diaphragm physiology, and we will examine correction of histopathology. Thus, preliminary studies suggest that gene replacement for desminopathy is a promising treatment strategy.

KEYNOTE 3: GENE THERAPY FOR PRIMARY IMMUNODEFICIENCIES

Sponsored by Aspen Australia

Bobby Gaspar, Professor of Paediatrics and Immunology, Centre for Immunodeficiency, UCL Institute of Child Health/Great Ormond Street NHS Trust

Primary immunodeficiencies have played a major role in the development of gene therapy for monogenic diseases of the bone marrow. The last decade has seen convincing evidence of long term disease correction as a result of ex vivo viral vector mediated gene transfer into autologous haematopoietic stem cells.

The success of these early studies has been balanced by the development of vector related insertional mutagenic events. More recently the use of alternative vector designs with self inactivating (SIN) designs which have an improved safety profile has led to the initiation of a wave of new studies which are showing early signs of efficacy. These studies in SCID-X1, ADA-SCID, Wiskott-Aldrich syndrome and Chronic Granulomatous disease are all multi-centre studies using lentiviral vectors and have the potential to recruit patients rapidly and to show efficacy and safety. The ongoing development of safer vector platforms and gene editing technologies together with improvements in cell transduction techniques and optimised conditioning regimes is likely to make gene therapy amenable for a greater number of PIDs. If long term efficacy and safety are shown, gene therapy will become a standard treatment option for specific forms of PID. These technologies may also be important for other monogenic disorders of the haematopoietic system.

KEYNOTE 4: BENCH TO BEDSIDE TRANSITION OF GENE THERAPY FOR HAEMOPHILIA B

Edward Tuddenham via Teleconferencing facilities, Emeritus Professor of Haemophilia UCL and Katharine Dormandy Haemophilia Centre, UK

KEYNOTE 5: GENE AND CELL THERAPY FOR PULMONARY HYPERTENSION

Paul N Reynolds, Department of Thoracic Medicine, Lung Research Laboratory, Royal Adelaide Hospital and University of Adelaide, Adelaide, SA, Australia

Pulmonary Arterial Hypertension (PAH) is a fatal disease characterised by remodelling of the pulmonary arterioles leading to right heart failure. Despite modern pharmaceuticals, mortality remains high at approximately 50% at 2 years in some studies. A variety of gene and cell-based approaches have been used to alter the remodelling process and ameliorate PAH, with clinical trials having been conducted using endothelial progenitor cells (EPCs) either de novo or transduced with the gene for endothelial nitric oxide synthase. PAH is causally related to mutations in the gene for Bone Morphogenetic Protein Receptor type 2 (BMPR2). BMPR2 is also downregulated in secondary forms of pulmonary hypertension (eg due to congenital heart disease) and in common animal models of PAH (rat monocrotaline and hypoxia). We thus hypothesised that upregulation of BMPR2 by gene transfer to the pulmonary vasculature in vivo would ameliorate PAH. We developed an adenoviral vector selectively targeted to the pulmonary vascular endothelium using a bi-specific conjugate, and used this to upregulate BMPR2. This approach successfully ameliorated PAH in both the rat hypoxia and monocrotaline models (reduced pulmonary artery pressures, reduced right ventricular hypertrophy and increased cardiac output). BMPR2 upragulation in human cells in vitro was associated with a decrease in smad 2/3 phosphorylation and an increased in smad 1/5/8 phosphorylation consistent with a shift from TGFb signalling to BMPR2 signalling. This shift was also associated with reduced endothelial to mesenchymal transition. We are now examining the effects of BMPR2 transduction on EPCs derived from PAH patients with a view to developing a new clinical treatment. Concurrently we are also developing improved adenoviral vectors with better targeting potential for in vivo transduction. Our approaches represent the first successful demonstration that BMPR2 modulation may be exploited for therapeutic purposes in PAH.

KEYNOTE 6: STEM CELLS AND REGENERATIVE MEDICINE: THE FUTURE IS NOW

Martin F. Pera, University of Melbourne, The Florey Neuroscience and Mental Health Institute, and the Walter and Eliza Hall Institute of Medical Research

The past decade has witnessed exponential growth in the fields of stem cell biology and regenerative medicine. Cell therapy is now a billion dollar industry, Thousands of clinical trials of mesenchymal stem cells for diverse indications have been undertaken. Trials of embryonic stem (ES) cell derived therapeutics are underway for macular degeneration, and ES products for other applications including Type 1 diabetes are in the pipeline. The discovery of induced pluripotency has opened up a new era in human functional genomics, disease modeling, and drug discovery. In this lecture we will survey the remarkable progress to date in this field and highlight the challenges that lie ahead. In particular I will highlight obstacles to clinical translation in this field relating to the mechanism of action and safety of stem cell derived grafts in patients.

The Greg Johnson Memorial Oration

GENETIC BASIS OF TELOMERE DISORDERS

Roger Reddel, Christine Napier, Kylie Bower, Jane Noble, Lily Huschtscha, Rebecca Dagg, Loretta Lau, Michael Stutz and Hilda Pickett, Cancer Research Unit, Children's Medical Research Institute, Westmead, NSW 2145

The cell division cycle of normal human somatic cells is accompanied by telomere shortening, with sets an upper limit on cellular proliferative capacity. In highly proliferative tissues, such as the bone marrow, this is partially counteracted by a telomere lengthening mechanism (TLM), primarily the enzyme telomerase which reverse transcribes new telomeric sequence. There is a growing list of clinical disorders associated with excessive telomere shortening. Common features include bone marrow failure, disorders of skin and mucous membranes, and pulmonary fibrosis, but a wide variety of organs may be affected and in severe cases there may also be developmental defects. Known genetic causes of short telomere syndromes include mutations in any of the genes that encode subunits of the telomerase enzyme ribonucleoprotein complex, proteins involved in its biogenesis, a protein required for its trafficking to the telomere, a protein involved in processing telomere ends, or a component of the shelterin telomere-binding protein complex.

Cancer is also a telomeric disease: almost all cancers have an upregulated TLM which completely counteracts normal telomere shortening thus permitting unlimited proliferation. The upregulated TLM is telomerase in 85% of cancers and Alternative Lengthening of Telomeres (ALT) mechanism in most of the remainder. How these TLMs become upregulated is incompletely understood, but activation of telomerase is commonly associated with a mutation in the promoter for the gene encoding its reverse transcriptase subunit, TERT. Activation of ALT is often associated with mutations in the ATRX gene which encodes a protein involved in chromatin remodelling. This may present opportunities for therapeutic intervention.

KEYNOTE 7: GENE THERAPY STRATEGIES FOR GENETIC LEUKODYSTROPHIES

Nathalie Cartier, INSERM, Paris, FRANCE

ORAL PRESENTATIONS

O1: ANALYSIS AND COMPARISON OF γ-RETROVIRAL VECTOR INTEGRATION BEHAVIOUR IN HUMAN CD34+ CELLS TRANSDUCED UNDER CONDITIONS EMPLOYED IN THE FRENCH AND ENGLISH SCID-X1 CLINICAL TRIALS

Claus V. Hallwirth[1], Gagan Garg[2], Nirav Malani[3], Tim Peters[4], Nicola Hetherington[1], Jessica Hyman[5], Belinda Kramer[5], Samantha L. Ginn[1], Xiaoan Ruan[6], Shoba Ranganathan[2], Michael Buckley[4], Frederic D. Bushman[3] and Ian E. Alexander[1,7], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, NSW, Australia; [2]Department of

Chemistry and Biomolecular Sciences, Macquarie University, Macquarie Park, NSW, Australia; [3]University of Pennsylvania School of Medicine, Department of Microbiology, Philadelphia, Pennsylvania, USA; [4]Division of Mathematics, Informatics and Statistics, CSIRO, North Ryde, NSW, Australia; [5]Children's Cancer Research Unit, The Children's Hospital at Westmead, Westmead, NSW, Australia; [6]Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore; [7]The University of Sydney, Discipline of Paediatrics and Child Health, Westmead, NSW, Australia

Genotoxicity resulting from vector-mediated insertional mutagenesis currently presents the most pressing challenge in the development of gene therapy protocols targeting diseases of the haematopoietic compartment. Since the discovery of pronounced vector integration site clustering in transduced cells prior to their implantation and potential *in vivo* selection, the development of safer gene therapy protocols increasingly relies on the determination and analysis of vector integration patterns in cells experimentally transduced under conditions of potential relevance to clinical trials. In this study, human $CD34^+$ progenitor cells were transduced under conditions reflecting those employed in the SCID-X1 clinical trials conducted in France (2000) and England (2002). French transduction conditions were found to be more pro-proliferative with reference to the bulk cell population transduced, and CD34-positivity was differentially retained during transduction. These observations, together with the fact that four of ten participants in the French SCID-X1 trial developed leukaemia, compared with only one of ten in the English trial, warranted consideration of the possibility that the different transduction conditions could result in recognisably disparate vector integration patterns. To address this question, vector/genomic DNA junction fragment libraries were generated from the transduced cells, using a modified ligation-mediated PCR approach, coupled to Illumina next-generation sequencing (NGS). The resulting datasets were sufficiently complex to facilitate several statistical comparisons of integration behaviour that have not previously been employed. Furthermore, analyses of the integration site datasets have identified the need to bioinformatically discern true integration sites from false positives, the latter likely arising from artefactual reads generated during NGS.

O2: STRESS HAEMATOPOIESIS AND MALIGNANCY: IMPLICATIONS FOR EMERGING GENE AND CELL-BASED THERAPIES

Samantha L. Ginn[1,2], Sophia H.Y. Liao[1], Claus V. Hallwirth[1], Erdahl T. Teber[3,4], Roger R. Reddel[5], Matthew McCormack[6], Adrian J. Thrasher[7], Min Hu[8], Steven I. Alexander[8] and Ian E. Alexander[1,9], [1]Gene Therapy Research Unit of the Children's Medical Research Institute and The Children's Hospital at Westmead; [2]The University of Sydney, Sydney Medical School; [3]Bioinformatics Unit of the Children's Medical Research Institute; [4]The Kids Cancer Alliance; [5]Cancer Research Unit of the Children's Medical Research Institute; [6] Cancer and Haematology Division, The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia; [7]Molecular Immunology Unit, UCL Institute of Child Health, London; [8]Centre for Kidney Research of The Children's Hospital at Westmead and [9]The University of Sydney, Discipline of Paediatrics and Child Health. Westmead, NSW, Australia

We have previously identified a relationship between haematopoietic progenitor cell dose and the incidence of lymphoid malignancy in the SCID-X1 mouse when undergoing immunological reconstitution[1]. We hypothesised that this was caused by replicative stress resulting from the progenitors being driven to undergo abnormal levels of replication when cell dose was limiting. In the current study, we sought to further investigate this relationship, and to correlate this with the levels of cellular proliferation occurring during lymphopoiesis by limiting cell dose.

Between 6.6 and 17.6 months post-transplantation, lymphomas were detected exclusively in mice from the mid- and low-dose treatment groups. Comparison of the survival of these two cohorts of mice with those receiving the high cell dose demonstrates that there was a significant difference in the lymphoma-free survival rates of these two groups ($P = 0.0323$ and $P = 0.0001$, respectively). Both tumour incidence and latency directly correlated with cell dose, which is consistent with our original hypothesis. In order to further examine cellular proliferation during lymphopoiesis, we have analysed lymphoid tissues for the presence of T cell receptor excision circles, their T cell receptor V_β repertoire and spectratype and level of Ki-67 expression. Collectively, the data generated confirm the presence of increased proliferative demands on progenitor cells after low dose reconstitution and reveals an unexplored risk factor for the development of malignancy inherent in contemporary gene therapy protocols targeting the haematopoietic system when reconstitution occurs from an unusually low number of progenitors. In addition, this has broader implications for contemporary gene and cell-based protocols that require non-physiological levels of replicative expansion that include gene correction strategies and the use of induced pluripotent stem cells.

[1]Ginn, S.L., *et al.* (2010). *Molecular Therapy* **18(5):** 965-976

O3: IMPACT OF NEXT GENERATION SEQUENCING ERROR ON THE ANALYSIS OF BARCODED PLASMID LIBRARIES OF DEFINED COMPLEXITY COMPRISING KNOWN SEQUENCE IDENTITIES

Claire T. Deakin[1], Jeffrey J. Deakin[1], Claus V. Hallwirth[1], Samantha L. Ginn[1], Paul Young[2], David Humphries[2], Catherine M. Suter[2] and Ian E. Alexander[1], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, New South Wales, 2145, Australia; [2]Molecular Genetics Division, Victor Chang Cardiac Research Institute, Sydney, Darlinghurst, New South Wales, 2010, Australia

Barcoded vectors are promising tools for analysing clonal diversity in haematopoietic gene therapy. Analysing haematopoietic clones marked with barcoded vectors requires an ability to accurately identify large numbers of barcode variants using next generation sequencing (NGS), when the exact number and sequence identity of barcodes is unknown. Given the potential for NGS error, the feasibility of NGS for this challenging application is unknown. The impact of sequencing error was investigated empirically, using barcode libraries comprising 1, 10 and 100 known sequences. Libraries containing 100 barcodes were sequenced with 4-5 log-fold coverage on the Illumina and SOLiD platforms. Libraries containing 1 and 10 barcodes could readily be distinguished from false barcodes generated by sequencing error by 3-4 log-fold. For the libraries containing 100 barcodes, however, there was an overlap between expected and false barcodes. GC content and likelihood of secondary structure formation did not predict the discrepancy between actual and observed barcode abundances, with 2-3 barcodes undetected. Furthermore, systematic sequencing errors were identified and were reproducible in independent Illumina sequencing runs. This analysis has uncovered limitations to the resolvable degree of barcode complexity, the sensitivity and specificity of barcode detection, and the quantitative potential for estimating clone size using barcodes.

O4: DEVELOPMENT AND VALIDATION OF MODEL SYSTEMS FOR THE INVESTIGATION OF RED BLOOD CELL DEVELOPMENT AND DISEASE

Jim Vadolas[1], Betty Kao[1], Mark Roosjen[1,2,] Orane Delagneau[1] and Bradley McColl[1], [1]Cell and Gene Therapy Research group, Murdoch Childrens Research institute, Royal Children's Hospital, Parkville, Victoria, Australia, [2]Van

Hall Larenstein University of Applied Sciences, Leeuwarden, The Netherlands

Haematopoiesis during mammalian development is characterised by the progressive appearance of distinct populations of cells at stage-specific sites within the developing embryo. The earliest "primitive" haematopoietic cells appear in the yolk sac, before being later replaced by "definitive" cells, located first in the aorta-gonad mesenephros, then the foetal liver and finally in bone marrow of the adult. In concert with the progression from primitive to definitive haematopoiesis, the developing erythroid system expresses developmentally-specific forms of haemoglobin, in a process known as haemoglobin switching. The pattern of globin gene expression during development is the result of a complex series of regulatory events. Numerous epigenetic and transcriptional regulators are required for switching to occur, but the process remains incompletely understood, despite several decades of research. Study of haemoglobin switching has been stimulated by recognition of the therapeutic potential of globin switching in the setting of the inherited β-haemoglobinopathies. Hereditary persistence of foetal haemoglobin (HPFH) is a non-pathological condition in which the silencing of the foetal γ-globin gene is reduced or absent, resulting in the persistent expression γ-globin after birth. Co-inheritance of HPFH with β-thalassaemia or sickle cell disease genotypes results in a milder symptoms, due to complementation of the mutant β-globin by the γ-globin. Understanding the process whereby γ-globin is silenced is therefore of clinical significance, with the ultimate goal of identifying therapeutic strategies capable of reactivating γ-globin expression in the adult. This aim has been the driving force behind the generation of several *in vitro* and *in vivo* model systems that recapitulate the human foetal to adult haemoglobin expression pattern throughout development and disease.

O5: EPIGENETIC THERAPY FOR β-THALASSEMIA

Betty Kao[1], L. Jamie Gearing[2], Miha Pakusch[2], Tracy Willson[2], Orane Delagneau[1], Mark Roosjen[1], Bradley McColl[1], Marnie Blewitt[2] and Jim Vadolas[1], [1]Cell and Gene Therapy Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, Parkville, VIC 3052, Australia. [2]Division of Molecular Medicine, Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville, VIC 3052, Australia

Gene silencing by RNA interference (RNAi) is a valuable tool for investigating gene function that also possesses great potential as therapy for genetic disorders. β-thalassemia is a genetic disorder caused by insufficient production of the β-globin subunit of hemoglobin. Reactivation of developmentally silenced γ-globin can ameliorate the clinical symptoms of β-thalassemia and is therefore a potential therapy. The mechanism by which γ-globin is developmentally silenced has not been fully elucidated although epigenetic regulation is known to play a vital role. This research aims to identify novel epigenetic enzymes that control γ-globin silencing and subsequently silence these genes by RNAi to reactivate γ-globin as a therapy for β-thalassemia. We have developed an assay system for the identification of novel epigenetic enzymes that control γ-globin silencing. This system involves the use a bespoke shRNA library that targets known and putative epigenetic enzymes in conjunction with our unique γ-globin-GFP reporter mouse. The miR-30 based shRNAs will be virally delivered into primary erythroid cells derived from the reporter mouse and reactivation of γ-globin assayed as increased GFP by flow cytometry. Validation of this system was carried out using shRNAs targeting *Dnmt1*, an epigenetic enzyme known to play a role in γ-globin silencing. Subsequent experiments will evaluate the therapeutic potential of the shRNAs *in vivo* in β-thalassemia mouse models and *in vitro* in primary erythroid culture of β-thalassemia patient cells. This research will contribute towards greater understanding of γ-globin silencing and lay the foundations for the development of RNAi as a therapy for β-thalassemia.

O6: CELL-DELIVERED GENE THERAPY FOR HIV

Geoff Symonds, St Vincent's Centre for Applied Medical Research and Calimmune Pty Ltd, Sydney, Australia

HIV is a disease not solved; while classical anti-retroviral therapy (ART) has the ability to reduce viral load and maintain CD4 count thereby reducing morbidity and mortality, it must be taken daily lifelong, meaning that there are cumulative side effects that can be severe and resistance can develop due to compliance issues. It is not curative due to the long-term maintenance of a viral reservoir. Cell-delivered HIV gene therapy involves the introduction of anti-HIV genes into blood cells to provide a population of protected cells that inhibit HIV replication, reduce the viral reservoir size and maintain CD4 counts. It has the potential to reduce or eliminate the need for ART. The work presented describes the use of a self-inactivating lentiviral vector containing a short hairpin RNA to the HIV co-receptor CCR5 and a fusion inhibitor. This dual vector shows robust expression in blood cells and strong and sustained inhibition of HIV replication. The progress of this work to clinical trial will be described. A recent HIV positive patient – the so-called "Berlin patient" in whom HIV was functionally cured by allogeneic transplant of blood stem cells containing mutant CCR5 (co-receptor for HIV) - points to the ability to "cure" HIV.

O7: RESULTS FROM A PHASE I/II CLINICAL TRIAL ON ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR GENE THERAPY IN PATIENTS WITH EXUDATIVE AGE-RELATED MACULAR DEGENERATION

Chooi-May Lai[1], Aaron Magno[2], Cora Pierce[2], Richard J Samulski[3], Thomas W Chalberg[4], Mark S Blumenkranz[5], Ian J Constable[1] and Elizabeth P Rakoczy[1], [1]Centre for Ophthalmology and Visual Science, The University of Western Australia, Crawley, Western Australia, Australia, [2]Lions Eye Institute, Nedlands, Western Australia, Australia; [3]Department of Pharmacology and Gene Therapy Centre, University of North Carolina, Chapel Hill, North Carolina, U.S.A; [4]Avalanche Biotechnologies, 665 Third St Suite 250, San Francisco, California, U.S.A; [5]Byers Eye Institute at Stanford, Palo Alto, California, U.S.A.

Age-Related Macular Degeneration **(**AMD) is the leading cause of vision loss in the elderly. We report here the safety of rAAV.sFlt-1 one year after subretinal injection into exudative AMD patients in a Phase I/II clinical trial. Eight patients (mean age: 79.0±4.6 years) with longstanding and extensively treated exudative AMD were randomized to treatment with low-dose (10^{10} vg, n=3) or high-dose (10^{11} vg, n=3) rAAV.sFlt-1 or to control (n=2). At baseline (day 0) and day 30, all patients received 0.5 mg ranibizumab to provide anti-vascular endothelial growth factor therapy during the initial ramp-up period. At day 7, the treatment group received low- or high-dose rAAV.sFlt-1 administered in 100-ul volume. Subretinal injection was successfully performed in all six patients. Ophthalmic examinations over time did not detect any significant intraocular pressure elevation, retinal detachment, or significant intraocular or systemic inflammation in any patient. The rAAV.sFlt-1 vector sequence was detected in the tear specimen from the injected eye of two patients at one day post-injection but not at any subsequent sampling timepoints or in any other specimens (saliva, urine, plasma/serum). Clinical laboratory assessments showed no significant changes from baseline. The presence of anti-AAV2 neutralising antibodies in two rAAV.sFlt-1-injected patients at baseline did not appear to affect safety. There were apparent visual acuity gains and a reduction in as-needed ranibizumab injection frequency over the 1-year follow-up. These initial results suggest that subretinal injection of rAAV.sFlt-1 is safe and well-tolerated, and is not associated with systemic or ophthalmic complications even among the elderly.

O8: INITIATION OF A PHASE I CANCER GENE THERAPY TRIAL AT THE CHILDREN'S HOSPITAL AT

WESTMEAD

Belinda Kramer[1], Rebecca Dent[1], Radhika Singh[1], Amanda Rush[1], Kimberley Lilischkis[2], Vicki Antonenas[3], David Gottlieb[3], Peter Shaw[2], Ian Alexander[4,5] and Geoff McCowage[2], [1]Children's Cancer Research Unit, Kid's Research Institute, The Children's Hospital at Westmead; [2]Oncology Unit, the Children's Hospital at Westmead; [3]Sydney Cellular Therapies Laboratory, Westmead Hospital; [4]Gene Therapy Research Unit of the Children's Medical Research Institute and The Children's Hospital at Westmead; [5]The University of Sydney, Discipline of Paediatrics and Child Health. Westmead, NSW, Australia.

A clinical trial to test the feasibility and safety of a gene therapy strategy for the treatment of brain tumours in children has commenced at the Children's Hospital at Westmead. This trial is open to patients with tumours that have relapsed following conventional treatment, or patients with a newly diagnosed brain stem glioma, which have very poor prognosis with current treatments.

Paediatric brain tumours can express high levels of endogenous Methyl-Guanine-Methyl-Transferase (MGMT), a DNA repair protein that renders cells resistant to methylating chemotherapy and dose escalation to overcome resistance is not possible due to accompanying dose-limiting toxicity in the bone marrow.

The strategy being trialled aims to confer chemotherapy resistance (chemo-protection) to Haematopoietic Stem or Progenitor Cells (HSC / HPC) using retroviral gene transfer of a mutant MGMT (MGMT(P140K)) to the haematopoietic compartment. The gene-transfer protocol involves 5 days of *ex vivo* culture of G-CSF Mobilised, $CD34^+$ selected Peripheral Blood Stem Cells (PBSC), during which the patient receives non-myeloablative conditioning, followed by infusion of the gene-modified cells. Further cycles of chemotherapy aimed at treating the tumour and selecting for gene-modified cells are then given at 3 – 4 weekly intervals, with the goal of achieving dose escalation.

Two patients have been enrolled on the study to date, with successful mobilisation of PBSC, gene transfer processing and re-infusion of gene-modified $CD34^+$ HSC/HPC. Detection of gene-modified cells post infusion has been at low levels. One patient was moved off-treatment as a result of tumour progression, while the second has received 5 cycles of post-therapy chemotherapy, with no evidence of tumour progression.

O9: MINIMALLY INVASIVE REPAIR OF ORTHOPAEDIC IMPLANTS USING GENE THERAPY

Gerald W Both, Andrew M Bray, Jolanda J De Poorter* and Rob GHH Nelissen*, Elk Orthobiologics Limited, Hawthorn East, Victoria and *Department of Orthopedics, Leiden University Medical Center, The Netherlands

The implantation of artificial knees and hips to overcome degenerative conditions is now commonplace but up to 10% of hip and knee prostheses fail within 10 years of the primary implantation due to the development of aseptic loosening. In this painful and debilitating condition bone is replaced by a soft inflamed tissue over time, resulting in the loss of implant stability. Patients who suffer from co-morbidities that make them unsuitable for revision surgery currently have no treatment options. They are left in pain with restricted mobility and often become bed-ridden. Due to the increasing number of operations being performed and the complications emerging from some previous use of unsuitable materials, particularly for hip implants, this problem is set to worsen in future years. A minimally-invasive, needle-based procedure has been developed to address aseptic loosening of hip implants. An adenoviral vector that expresses bacterial nitroreductase (NTR) is injected to infect the interface tissue in the hip cavity. After two days a pro-drug (an alkylating agent, CB1954) is also injected. This is converted to a DNA cross-linking agent by NTR, resulting in destruction of the interface tissue which is removed by flushing the peri-prosthetic space with EDTA solution. The prosthesis is then

repositioned prior to the injection of bone cement to complete the repair. The safety of the procedure and the enzyme/pro-drug system has been demonstrated in a phase I trial at Leiden University Medical Center, The Netherlands. The approach is likely to be applicable to the repair of other orthopaedic implants, particularly knees.

O10: TARGETED DELIVERY OF RRM1-SPECIFIC SIRNA LEADS TO TUMOUR GROWTH INHIBITION IN MALIGNANT PLEURAL MESOTHELIOMA

Glen Reid[1], Marissa Williams[1], Michaela B Kirschner[1], Yuen Yee Cheng[1], Nancy Mugridge[2], Jocelyn Weiss[2], Sonja Klebe[3], Himanshu Brahmbhatt[2], Jennifer MacDiarmid[2], Nico van Zandwijk[1], [1]Asbestos Diseases Research Institute, University of Sydney, Sydney, Australia; [2]EnGeneIC, Sydney, Australia; [3]Flinders University, Adelaide, South Australia

Malignant pleural mesothelioma (MPM) is recalcitrant to treatment and new treatments are urgently needed. Multiple genes involved in proliferation and metabolic activity are upregulated in MPM and these represent attractive targets for an siRNA-based intervention. We carried out an RNAi-based screen of 40 target genes to identify candidate genes with roles in cell growth and survival in MPM cells. All 40 genes were effectively silenced, and for 6 genes knockdown with 2 independent siRNAs resulted in significant growth inhibition over time in multiple cell lines: PLK1, CDK1, NDC80, RRM1, RRM2 and BIRC5 (Survivin). Dose response experiments with varying concentrations of siRNA revealed that siRNAs specific for RRM1 and RRM2 were the most effective at inhibiting growth with IC50 values in the low nanomolar range. A chemically modified siRNA targeting RRM1 was selected for in vivo studies in a xenograft model of MPM. Intravenous administration of RRM1 siRNA packaged in minicells targeted with EGFR-specific antibodies ($2x10^9$ minicells per dose, 4 times per week for 3 weeks) led to consistent and dose-dependent inhibition of MPM tumor growth compared with treatment with an inactive siRNA. Reducing the dose and number of administrations did not reduce growth inhibition; as little as $1x10^9$ minicells administered once a week were sufficient to completely inhibit MPM tumour growth. In conclusion, RRM1 is an attractive target for siRNA-based inhibition, and siRNA delivery with EGFR-targeted minicells represents a novel therapeutic approach for MPM.

O11: MICRORNAS AS NOVEL ANTI-ANGIOGENESIS THERAPEUTIC TARGETS

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Tumour angiogenesis is essential for malignant growth and metastasis. Targeting of this process can stop tumour growth, however current anti-angiogenesis therapies have severe side-effects and only prolong the life of patients for a short time due to adaptive resistance. Bone marrow (BM)-derived endothelial progenitor cells (EPCs) contribute to angiogenesis-mediated tumour growth. We have demonstrated that EPCs can be tracked from the BM to sites throughout the body

including the BM compartment of the tumour-stroma[1,2]. Here we report through the use of transgenic mouse models, lentiviral delivery of transgenes *in vivo* and microRNA deep sequencing, a novel small RNA pathway required for regulation of EPC function and tumour angiogenesis. In this pathway, the microRNA 10b is up-regulated in tumour vasculature and EPCs in response to tumour challenge. Using fluorescent *in situ* hybridization of human breast cancer biopsies we determined that the microRNA 10b is expressed in the vasculature of aggressive late stage invasive ductal carcinoma. We have also shown that this microRNA can be targeted *in vivo* using an anti-miR encapsulated in a liposome. This resulted in decreased tumour growth, vasculature development and EPC mobilization[2]. We are currently altering this system so that the liposome will be conjugated with a novel EPC peptide so that the anti-miR will be delivered directly to EPCs. As this approach would target tumour angiogenesis but not normal vasculature, due to microRNA 10b expression patterns, we believe this approach may lead to a novel anti-cancer therapy without problems associated with current therapies.

(1) Plummer PN, Freeman R, Taft R et al. MicroRNAs Regulate Tumor Angiogenesis Modulated by Endothelial Progenitor Cells. Cancer Research (2013) 73:341-52

(2) Mellick AS, Plummer PN, Nolan D, et al. Using Transcription Factor Id1 to Selectively Target Endothelial Progenitor Cells Offers Novel Strategies to Inhibit Tumour Angiogenesis and Growth. Cancer Research (2010) 70:7273-82.

O12: NANOPARTICLE DELIVERY OF RNA-BASED THERAPY TO MODULATE TUMOUR VASCULATURE: AS A NEXT GENERATION ANTICANCER THERAPY

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For cancers to grow and spread they need to enroll pre-existing vasculature in a process referred to as angiogenesis. If you can block this process you can greatly increase the survival of patients suffering from solid tumours. Such therapies as Avastin block angiogenesis by interrupting vascular endothelial growth factor (VEGF)-VEGF receptor (VEGFR) signaling, but have shown limited efficacy because of the advent of adaptive resistance. There are several proposed cellular and molecular mechanisms proposed for the advent of adaptive resistance. In the last ten years, our laboratory has focused on the role of bone marrow derived endothelial progenitor cells (EPCs)[1], in promoting tumour growth by assisting in the process of angiogenesis. EPCs promote vascular development by producing paracrine pro-angiogenic factors, and by directly incorporating into tumour vasculature. Both tumour EPCs and tumour vasculature show different gene expression signatures to normal EPCs and normal endothelial cells. This is best demonstrated by the differential expression of small noncoding (micro) RNAs. We have used these differences to deliver RNA-based therapy (RBT) to modulate microRNA function in EPCs and tumour vasculature, using nanoparticle (liposome) based delivery of anti-miRNAs. The results published recently in *Cancer research* (2013)[2], provide the basis for next generation gene and cell based therapies.

(1) Mellick AS, Plummer P, Nolan D, et al. Using Transcription Factor Id1 to Selectively Target Endothelial Progenitor Cells Offers Novel Strategies to Inhibit Tumor Angiogenesis and Growth. *Cancer Res*. 70:7273-82, 2010.

(2) Plummer PN, Freeman R, Taft R, Vider J, Sax M, Umer BA, Gao D, Johns CA, Mattick JS, Wilton SD, Ferro V, McMillan NA, Swarbrick A, Mittal V, Mellick AS. MicroRNAs regulate tumor angiogenesis

modulated by endothelial progenitor cells. *Cancer Res*. 73:341-52,2013.

O13: STEM CELL-BASED DELIVERY OF SUICIDE GENE YCDUPRT THERAPY TO PROSTATE CANCER

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One in nine men will be diagnosed with prostate cancer (PCa) in their lifetime. Bone marrow derived mesenchymal stem cells (BMSC) show promise as therapeutic gene transfer vehicles due to their ability to migrate to tumour sites anywhere in the body while evading rejection by the immune system, a major hurdle for most gene delivery systems currently under investigation.

BMSCs were nucleofected with the *in vitro* and *in vivo* cell tracking reporter gene firefly luciferase (fl) and the yeast fusion suicide gene cytosine deaminase and uracil phosphotransferase (BMSC-yCDUPRT) *ex vivo.* yCDUPRT converts 5FC to the toxic metabolite 5-fluorouracil (5FU) which leads to cancer cell death. RM1 prostate cancer cells (RM1) were gene modified with another *in vitro* and *in vivo* cell racking reporter gene renilla luciferase (rl). In this study we used the syngeneic and immune intact albino C57BL/6 mouse model for tracking BMSC efficacy *in vivo*.

Mice were implanted subcutaneously with either RM1 cells alone or a mixture of RM1/BMSC-yCDUPRT cells and tumours were allowed to establish. Mice were then treated systemically with 5FC or saline for 10 days. Sequential IVIS Lumina II bioluminescence full body imaging (BLI; Caliper/ Thermo Scientific) was used to quantify and track the effect of BMSC-yCDUPRT ± 5FC on RM1 tumour growth. Light reactions produced by RM1-rl substrate coelentrazine supported by tumour volume measurements showed a significant reduction in RM1 tumour growth in the presence of BMSC-yCDUPRT/5FC when compared to the control groups. Thus, BMSC-yCDUPRT/5FC showed comprehensive PCa tumour killing *in vitro* as well as *in vivo*.

O14: QUALITY ASPECTS OF A PHASE I GENE THERAPY TRIAL IN ONCOLOGY

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In June 2012, the Children's Hospital at Westmead opened a Phase I clinical trial to test the safety and feasibility of a gene therapy strategy for the treatment of brain tumours in paediatric patients. Regulatory approval for the trial was obtained via application to the TGA under the Clinical Trials Exemption (CTX) scheme, a process which resulted in mutual agreement over the cell processing procedures, critical materials and acceptance criteria for release of gene-modified cells.

From a laboratory perspective, the genetic modification of patient Haematopoietic stem cells (HSC) is carried out in a cleanroom facility under Standard Operating Procedures that govern facility infrastructure use, processing methodology and environmental monitoring. A comprehensive Quality System to underpin this activity has been instituted over the past 3 years in association with other cell therapy programs within Sydney Cell and Gene Therapy (SCGT) in an effort to co-ordinate effort and share the experience of groups working within the

cellular and gene therapy fields at Westmead.

In addition to the quality system required for the production of cellular products for use, their delivery to patients within the context of an HREC approved clinical trial also requires that a system be in place to ensure compliance Good Clinical Practice (GCP) guidelines.

The experience of having generated reagents and approved processes, submitted applications for regulatory approval and instituted quality systems for both laboratory activity and for the conduct of a clinical trial such as this has provided a template for future Phase I studies in oncology at The Children's Hospital at Westmead.

O15: BIONIC MATERIALS FOR NEUROMUSCULAR RESTORATION AND MAINTENANCE

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Effective engineering of skeletal muscle requires platforms that facilitate the proliferation and maintenance of primary muscle stem cells (myoblasts) and muscle fibre maturation in a manner that reflects native muscle structure. In addition, the supporting scaffold needs to accommodate the correct innervation of the re-engineered muscle tissue by promoting axonal connection and neuromuscular junction formation. We have been investigating the use of micro and nano-structured conducting polymer surfaces for ex vivo muscle and nerve growth, differentiation and trophic electrical stimulation. Micro-structured platforms were created by localization of wet-spun PLA:PLGA fibers onto polypyrrole substrate, whilst nano-structured platforms were created by orientation of carbon nanotube fibres on a conducting gold mylar surface, over which a layers of polypyrrole were deposited. Human and murine myoblasts and rat dorsal root ganglion explants (sensory nerve) were grown and/or differentiated on these platforms. A significant effect on myotube orientation was seen on both micro and nano-structured surfaces whilst surface topography similarly influenced the direction in which elements of the DRG cellular components grew. Growth of muscle cells as well as sensory nerve components (Scwhann cells and axons) on both nano and micro-structured polypyrrole was increased by electrical stimulation, providing a novel model system by which the effective innervation of regenerating muscle can be explored.

O16: BONE MORPHOGENETIC PROTEIN (BMP) SIGNALING IS A POSITIVE REGULATOR OF SKELETAL MUSCLE MASS

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The Transforming Growth Factor–beta (TGF-β) signaling network regulates skeletal muscle development and adaptation. Myostatin, activin and other TGF-β ligands stimulate intracellular Smad2/3 signaling to repress muscle growth, and promote catabolism. However, a role in muscle for the parallel signaling pathway comprising bone morphogenetic proteins (BMP), BMP receptors and the regulatory Smad proteins 1, 5 and 8 has not been defined. Here, we show that BMP signaling positively regulates muscle mass. Phosphorylation of Smad1/5 via over-expression of BMP7 in murine muscles stimulated myofibre hypertrophy via increased

protein anabolism, but hypertrophy was prevented via Smad1/5 inhibition. Blockade of mTOR-dependent anabolic signalling prevented BMP-mediated hypertrophy despite increased Smad1/5 phosphorylation, indicating that BMP-mediated muscle hypertrophy involves recruitment of mTOR-dependent processes. An examination of models of muscle wasting determined that BMP signaling was depressed in cancer cachexia, and conditions where myostatin and activin are elevated. In contrast, we observed that Smad1/5 phosphorylation was elevated in conditions of wasting associated with disruption of the neuromuscular junction (NMJ). Our data demonstrate that the BMP axis is stimulated in muscles following disruption of the NMJ as a mechanism to minimise muscle atrophy, as blockade of Smad1/5 in denervated muscles exacerbated muscle atrophy, whereas increased phosphorylation of Smad1/5 was protective. Combined, our studies demonstrate a new role for BMP signaling as a positive regulator of skeletal muscle mass. Interventions that stimulate BMP-Smad1/5 signaling may have potential to ameliorate the pathology of muscle wasting in neuromuscular disorders and other conditions associated with disruption of the NMJ.

O17: COMBINATORIAL GENE THERAPY USING AAV VECTOR TECHNOLOGY TO TREAT DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder resulting from a mutation in the dystrophin gene. DMD causes a loss of ambulation and leads to premature death from cardiac and/or respiratory failure. Although Gregorevic *et al*. demonstrated that administration of recombinant adeno-associated viral vectors carrying a truncated dystrophin gene (microdystrophin) increased muscle strength and longevity of treated dystrophin-/-:utrophin-/- (double knockout: *dko*) mice compared to their untreated littermates, wild-type levels of strength and lifespan were not obtained. Alternate strategies that increase muscle mass and strength have subsequently been pursued. Follistatin binds and inhibits negative regulators of muscle mass and has been recently confirmed to increase muscle mass and strength after systemic delivery (Winbanks *et al.*, 2012). We tested the hypothesis that intramuscular co-delivery of follistatin with microdystrophin would ameliorate the dystrophic pathology to a greater extent than either gene delivered in isolation.

Four weeks after a single intramuscular delivery of follistatin, there was a negative correlation between muscle mass and the severity of the dystrophic pathology. Biochemical analysis revealed a reduction in follistatin expression in dystrophic muscles and over time, detectable follistatin vector diminished. We hypothesise that the loss of vector genomes is due to increased fibre turnover in severely dystrophic muscle. However, follistatin expression was higher in combinatorial treated *dko* muscles than in muscles only treated with follistatin. These findings demonstrate the capacity for follistatin to increase muscle mass in severely dystrophic muscle and highlights the potential for combinatorial gene therapy to ameliorate the dystrophic pathology.

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O18: REVERSAL OF DIABETES IN A PORCINE MODEL FOLLOWING LIVER-DIRECTED GENE THERAPY

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Type I diabetes (TID) mellitus resulting from the T-cell mediated autoimmune destruction of insulin-producing pancreatic β-cells is currently treated with insulin therapy following blood glucose monitoring. This treatment delays but does not eliminate microvascular and macrovascular complications associated with T1D. Gene therapy is a strategy being explored to cure TID by providing non β-cells the ability to secrete insulin in response to physiological concentrations of glucose.

This study focused on the reversal of diabetes in pancreatectomised Westran pigs and showed pancreatic differentiation in the liver. We developed a novel surgical technique[1] to express furin-cleavable human insulin (INS-FUR) in the livers of 9 pancreatectomised Westran pigs, followed by an intravenous glucose tolerance test (IVGTT) in animals showing low blood glucose levels. While transient reduction in blood glucose was detected in a number of animals, only one pig maintained normal fasting blood glucose levels for the period from 10-44 days (experimental endpoint). PCR analysis of the liver tissue revealed expression of several β-cell transcription factors including *Pdx1* and *Neurod1*, pancreatic hormones glucagon and somatostatin, but not endogenous pig insulin. Triple immunofluorescence staining showed extensive insulin expression, and a small amount of glucagon and somatostatin protein expression in the liver.

The absence of endogenous pig insulin expression indicates that normoglycaemia was maintained by the expression of INS-FUR. This data verifies our previous work[2] and suggest that this regimen may ultimately be employed clinically to cure TID.

(1) Ren, B. *et al. Diabetologia* **50**, 1910-1920 (2007)

(2) Ren, B. *et al. J Gene Med* **15**, 28-41 (2013)

O19: GENERATION OF A MATHEMATICAL MODEL OF THE UREA CYCLE

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Current strategies for liver targeted gene therapy have mostly been designed to achieve expression of the deficient enzyme at normal levels, by maximising delivery of the transgene. However, disorders such as urea cycle defects show that partial ureagenic capacity is sufficient to ameliorate the disease phenotype. Partial correction may arise from either a small increase in urea cycle enzyme activity in all hepatocytes (e.g. 3% activity in 100% of cells), or a large increase in activity in only a subset of cells (100% activity in 3% of cells). To determine whether these two scenarios achieve the same level of therapeutic benefit, a two compartment mathematical model of the urea cycle was generated. The model predicted that partial gene correction in all cells is more beneficial than supraphysiological expression of the deficient enzyme in only a proportion of cells for gene therapy of ornithine transcarbamylase deficiency and citrullinaemia. The predictions were tested against experimental data and were

found to be similar to results observed from mouse models of urea cycle defects. The model predicted that correction of ASS deficiency by achieving gene correction in approximately 30% of cells would be required to approach normal levels of ammonia handling in citrullinaemia. This was similar to the 25% threshold which was observed in ASS knock-out mice after neonatal gene therapy. Overall, the mathematical model is a useful tool which can be used to approximate the minimal levels of gene transfer required to correct urea cycle defects.

O20: ADDRESSING THE CHALLENGE OF ACHIEVING STABLE rAAV-MEDIATED GENE TRANSFER IN THE GROWING LIVER

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Recombinant adeno-associated viral vectors (rAAVs) are currently the most promising delivery system for gene therapy targeting the adult liver. We are interested in extending this promise to the pediatric population however a major translational challenge of early gene therapy with rAAV is the maintenance of therapeutically adequate levels of transgene expression during liver growth. AAV vectors exist largely as episomes which are rapidly lost from proliferating hepatocytes, with only a small subset of cells remaining stably transduced as a consequence of genomic integration. We investigated whether this subset of integrated vector genomes following perinatal delivery was sufficient to protect against lethal hyperammonaemia in two independent models of urea cycle deficiency, the ASS knockout (ASS-KO) mouse and the partially OTC-deficient *Spf*^{ash} mouse rendered hyperammonaemic by shRNA-mediated knockdown of residual endogenous OTC activity. In both models a single dose of vector perinatally was insufficient to protect against hyperammonaemia. Additional vector doses were required for survival to adulthood in the ASS-KO mouse, achieving stable transduction of ~20% of hepatocytes expressing ASS at near physiological levels. We are currently developing a hybrid AAV/transposon vector system capable of conferring single-dose life-long phenotype correction based on piggyBac transposase.

O21: RECOMBINANT ADENO-ASSOCIATED VIRAL VECTOR TRANSDUCTION IS REDUCED IN THE PRESENCE OF CHRONIC LIVER DISEASE PATHOLOGY IN ABCB4-/- MICE

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The liver is an appealing target for gene therapy because the pathophysiology of many diseases arises from hepatocyte dysfunction. However, liver disease pathology including fibrosis, may impede vector delivery and transduction of hepatocytes, and thus, is an important consideration when designing strategies towards chronic liver diseases. We evaluated the impact of liver pathology on recombinant adeno-associated viral (rAAV) vector transduction using the Abcb4 knockout mouse, a model for Progressive Familial Intrahepatic Cholestasis type 3 in which juvenile mice develop progressive liver disease, leading to cirrhosis in early adulthood. Methods: Using an rAAV type 2 vector pseudo-serotyped with type 8 capsid encoding

eGFP, under the control of a hepatocyte-specific promoter/enhancer, we assessed transduction and GFP expression in $Abcb4^{-/-}$ and $Abcb4^{+/+}$ mice aged 0, 2, 4, 6, and 8 weeks. All mice received 2.5x10e11 vector genomes intraperitoneally. Blood and liver were obtained 1 week post-inoculation. Results: Histopathology showed progressive liver disease with age. Fibrosis was evident from 5 weeks, and cirrhosis detectable in young adults. There was a 1.7 to 15.7 fold reduction in vector copies per diploid genome in the liver of homozygotes compared to age-matched, disease-free, wild-types. GFP expression, measured by flurometry of liver protein lysates, was also comparably reduced in homozygotes. Fluorescence microscopy of homozygous livers showed reduced GFP expressing hepatocytes particularly within inflamed portal areas. Conclusion: In this PFIC3 model, liver pathology impeded rAAV vector delivery and transduction, thus necessitating initiation of rAAV gene therapy prior to onset of pathology in this and other chronic liver diseases.

O22: GENE THERAPY AS A TOOL FOR MANIPULATING THE IMMUNE SYSTEM TO PROMOTE IMMUNE TOLERANCE AND THE TREATMENT OF AUTOIMMUNITY

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Autoimmune diseases represent a class of pathologies in which the body's immune system has mounted a destructive immune response towards self-antigens. In Multiple Sclerosis, this is the myelin sheath surrounding neuronal axons. Treatment is often associated with long-term immunosuppression and a major challenge for new treatments is to provide a targeted therapy delivering disease specific immune tolerance. We know that exposure to self-antigens during lymphocyte development is central to inducing self-tolerance and that bone marrow (BM) compartment can have a key role in this. Over the years, we have developed a gene therapy strategy involving the *ex vivo* genetic manipulation of BM cells followed by transplantation to preconditioned recipients to promote antigen specific tolerance. We have used the mouse model of experimental autoimmune encephalomyelitis (EAE) as a model of multiple sclerosis and is induced by immunizing mice with myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}). Mice transplanted with BM transduced with retrovirus encoding MOG are not only resistant to the development of EAE but the strategy can also promote long-term remission in mice with established EAE. We have extended this and shown that tolerance and disease resistance can also be successfully accomplished with lower toxic non-myeloablative conditioning which reinforces clinical feasibility. Analysis of mechanisms demonstrates the process leads to antigen specific T and B cell deletion and that expression of antigen across a range of cell types can participate in this tolerance induction. We believe this gene therapy approach offers a strategy to promote immune tolerance for the treatment of autoimmune disease.

O23: BIODISTRIBUTION AND SAFETY PROFILE OF TRANSPLANTATED HUMAN MESENCHYMAL STROMAL CELLS IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Mesenchymal stromal/stem cells (MSCs) are being employed to treat inflammatory and degenerative diseases due their angiogenic, multipotent and anti-inflammatory properties. Whilst mechanistic studies have shown that MSCs produce a plethora of factors that promote tissue repair and suppress the immune response, their in vivo fate has yet to be clearly defined. To address this $1x10^6$ luciferase-expressing MSCs derived from human bone marrow (BM), adipose (Adi) and umbilical cord (UC) were administered systemically or in the peritoneum in Balb/c or NOD/Lt mice with experimental autoimmune encephalolomyelitis (EAE), respectively, and cells tracked using bioluminescent imaging. Following transplantation, MSCs injected in the venous system coalesced in the lungs where they persisted for 10-14 days. In mice with EAE, MSCs were observed for a similar time frame but were never detected in the central nervous system, which is the effected organ in this disease model. Despite the claim that MSCs are immune-privileged, this observation suggested that host immune-mediated mechanisms may have resulted in cell rejection. To test this Balb/c and EAE-NOD/Lt mice were transplanted with Adi-, BM- and UC-MSCs and immune responses measured. All animals generated robust antibody and T-cell responses indicating that MSCs are subject to immunological rejection. Serendipitously, when transplanted in immune-deficient NOD/SCID mice, only gene-modified BM-MSCs generated multiple macroscopic solid tumours with a transformed phenotype and karyotype. This comparative analysis demonstrates that whilst MSCs have a low efficiency to engraft, a thorough assessment of their immunological and oncogenic profile requires careful consideration prior their introduction into the clinic.

O24: PMO-MEDIATED DYSTROPHIN EXON 23 SKIPPING RESTORES MITOCHONDRIAL FUNCTION IN THE MDX HEART

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Approximately 20% of boys with Duchenne Muscular Dystrophy will die of dilated cardiomyopathy. The cardiomyopathy is characterised by disrupted structure and function of cardiac muscle cells and reduced energy production. However, the mechanisms responsible for the altered energy metabolism have been poorly understood. We have previously sought to identify the mechanisms for metabolic inhibition in *mdx* mouse cardiomyopathy. Calcium influx through the L-type Ca^{2+} channel (also known as the dihydropyridine receptor) in cardiac myocytes is essential for contraction. Calcium is also important for the regulation of mitochondrial function and production of ATP that is required to meet the energy demands of the heart. We have shown that the L-type Ca^{2+} channel can regulate mitochondrial function and metabolic activity in cardiac myocytes. In *mdx* heart, the communication between the L-type Ca^{2+} channel and the mitochondria is altered as a result of disruption of the cytoskeletal architecture. This contributes to metabolic inhibition in the *mdx* heart. We demonstrate that treatment of *mdx* mice with a phophorodiamidate morpholino oligomer, designed to induce skipping of exon 23, "restored" the increase in mitochondrial membrane potential in *mdx* myocytes after activation of the L-type Ca^{2+} channel with the dihydropyridine. These results confirm that metabolic inhibition occurs as a result of the absence of dystrophin, and oligomer therapy may be able to normalise metabolic activity and restore contractility in *mdx* mouse hearts.

O25: EXON SKIPPING AND DUCHENNE MUSCULAR DYSTROPHY: A CLINICAL TRIAL UPDATE

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The severe muscle wasting disease, Duchenne muscular dystrophy (DMD), arises from protein truncating mutations in the *DMD* gene that preclude synthesis of a functional protein. Antisense oligonucleotides have been designed to redirect dystrophin pre-mRNA processing so that an exon can be specifically excluded from the mature mRNA. We have developed a morpholino oligomer that excises dystrophin exon 51, and should restore functional dystrophin expression in the most common subset of DMD deletion patients. An extended placebo-controlled study was initiated in Columbus Ohio and this oligomer, now called Eteplirsen, has been administered intravenously to trial participants on a weekly basis at doses of 30mg/kg or 50 mg/kg. After 24 weeks administration, boys receiving Eteplirsen were unequivocally synthesizing dystrophin, but no clinically significant benefits were observed compared to the placebo group (rolled over to open label after 24 weeks). However, after 36 and 48 weeks treatment, statistically significant differences in the 6 minute walk test were seen in the treated groups, when compared to the placebo/delayed treatment cohort. The drug is well tolerated and has not been associated with any adverse effects. The trial is now being extended into late 2013. Additional oligomers are being designed to address different dystrophin mutations, and new clinical trials should be underway in 2013.

The promising DMD trial results have renewed enthusiasm to pursue splice intervention therapies for other disorders. An estimated 15% of human mutations induce aberrant splicing and splice switching oligomers may be used as a personalized genetic therapy, regardless of the mutated gene.

POSTER PRESENTATIONS

P1: PSEUDOGENE ACTIVATION IN THE DYSTROPHIN GENE OF A PATIENT WITH BECKER MUSCULAR DYSTROPHY

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We report an unusual mutation in the dystrophin gene in the form of a 58 base psuedoexon between exon 62 and 63 originating from the 3' end of intron 62. Interestingly, this mutation has been previously reported in another patient by a French group, who identified a SNP within intron 62 that activates a cryptic splice site, resulting in the retention of the pseudoexon. We are currently in the process of sequencing DNA from the patient to fully characterise the mutation. Another patient cell line with a 73 base pseudoexon in intron 47 has also been obtained. Antisense oligonucleotides (AOs) have been designed to excise these psuedoexons from the mature transcript. This would result in the transcription of a full-length normal dystrophin transcript, which is different to common exon skipping strategies that produce shorter transcripts. As such, these mutations may be particularly amenable to exon skipping.

P2: KNOCKDOWN OF PROXIMAL ENZYMES IN THE TYROSINE CATABOLIC PATHWAY BY SHRNA: A STRATEGY FOR IN VIVO EXPANSION OF GENE-MODIFIED HEPATOCYTES IN THE FAH DEFICIENT MOUSE MODEL

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Treatment of inborn errors of liver metabolism by AAV-mediated gene delivery where the corrected genetic defect does not confer a selective advantage poses a particular challenge in the growing liver. We and others have shown that AAV vector genomes are rapidly lost from cells undergoing proliferation, with only a small subset remaining that are stably transduced. Expansion of gene-modified cells by applying a selective pressure may be of benefit. In hereditary tyrosinaemia type I (HTI), characterised by a deficiency in fumaryl acetoacetate hydrolase (FAH), accumulation of fumaryl acetoacetate (FAA) causes hepatocyte death. In patients where mutant cells have reverted to wildtype, near-complete liver repopulation has been observed. Similarly, inhibiting enzymes upstream of FAH in tyrosine catabolism should also confer a selective advantage. In wildtype hepatocytes, the selective pressure of FAH deficiency can be mimicked with a small molecule inhibitor of FAH (CEHPOBA). We designed a panel of shRNA sequences targeting 4-hydroxyphenylpyruvate dioxygenase (HPD) and tyrosine aminotransferase (TAT). These were inserted into a rAAV2/8.eGFP under the transcriptional control of the H1 or U6 promoter, and injected into adult C57BL/6 mice. Vector constructs with the most efficient knockdown were then tested in FAH deficient mice. These mice are maintained on NTBC which blocks HPD and prevents FAA accumulation. Newborn mice were injected with $2.5x10^{11}$ vg of rAAV2/8.eGFP expressing shRNA and half were cycled off/on NTBC at 4 weeks post-injection. Clonal expansion of eGFP-expressing hepatocytes is currently being analysed. We propose to use this system in the treatment of urea cycle disorders.

P3: PROSTATE CANCER EXOSOMES OFFERING NOVEL CIRCULATING BIOMARKERS FOR EARLY CANCER DIAGNOSIS AND PROGNOSIS

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Prostate cancer (PCa) can be cured in approx. 80% of men presenting with early, organ confined disease following surgery to remove the prostate. The commonly used blood test for prostate-specific antigen (PSA) shows elevated serum levels in men with PCa. Unfortunately, PSA alone is a poor predictor of disease outcome (prognosis) and invasive prostate biopsies are required to determine the PCa stage and prognosis. As a result, poor testing compliance results in most men presenting with advanced disease which is largely incurable. Thus, we are investigating the utility of RNAs enriched in cellular nanovesicles (40-100nm in size) released into the circulation, to develop novel non-invasive biomarker tests for early PCa diagnosis and prognosis.

Total RNA was isolated from human prostate epithelial cells, established PCa cell lines and their culture supernatant exosomes. The microRNA (miRNA) and messenger RNA (mRNA) expression profiles from these sources were analysed using the Affymetrix and Arraystar microarray platforms and the data were mined using Partek Genomics Suite. Biomarker candidates were then validated using qPCR.

The expression profiles revealed a vast number of differentially expressed miRNAs and mRNAs in PCa exosomes that can be readily developed into potential biomarkers.

In brief, many miRNAs were up-regulated in PCa exosomes while the majority of mRNAs were down-regulated. Gene set analyses further suggested that differentially expressed exosomal RNAs were associated with various aspects of cancer development and progression. Exosomes show potential as sources for novel circulating biomarker discovery.

P4: ENGRAFTMENT, EXPANSION AND EVIDENCE OF MATURATION OF ADULT MURINE OVAL CELLS IN THE INJURED MOUSE LIVER

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Hepatocyte transplantation has been found to be beneficial in most of the 80 patients treated with metabolic liver disease or acute liver failure. However, hepatocytes are difficult to obtain and the search for alternative cell types for cell-based therapy of the liver is required. Liver progenitor cells, also known as oval cells in rodents, are a promising alternative to mature hepatocytes for many liver diseases. These cells are bipotent, being able to differentiate into both hepatocytes and biliary epithelial cells, and can engraft the liver and proliferate. Consequently, there is a need to better characterize oval cells in order to understand the factors controlling their activation, proliferation, differentiation and tumorigenic status. This project aims to undertake *in vivo* characterization of bipotential murine oval liver (BMOL) cells isolated from an adult mouse fed a choline-deficient, ethionine (CDE)-supplemented diet (Tirnitz-Parker *et al.,* 2007). These cells have undergone spontaneous immortalisation and have been previously characterized *in vitro*. Here, BMOL cells (1×10^6, 60% GFP^+) were injected intrasplenically into adult FRG mice and formed multiple clusters of up to 20 GFP^+ expressing cells after six weeks with characteristic hepatocyte morphology. Furthermore, engrafted cells expressed fumarylacetoacetate hydrolase (Fah), a marker of mature hepatocytes. In addition, no tumours were observed. Although our data is preliminary, to our knowledge it is the first evidence demonstrating that a murine oval cell line isolated from mice treated with a CDE-supplemented diet can be engrafted into the murine liver and undergo proliferation and maturation.

P5: TARGETED EXON SKIPPING TO CORRECT DUPLICATED EXONS IN THE DYSTROPHIN GENE

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Duchenne Muscular Dystrophy (DMD) is a severe muscle wasting disease caused by protein truncating mutations in the dystrophin gene that lead to the absence of a functional protein. Although exonic deletions are the most common type of DMD lesion, whole exon duplications can also disrupt the reading frame. Duplications account for 10-15% of all reported DMD mutations, and exon 2 is the most commonly duplicated exon. Here we describe *in vitro* evaluation of two different splice switching antisense oligomer chemistries and three distinct splice switching strategies to restore the dystrophin reading frame in DMD patient cells carrying a dystrophin exon 2 duplication. Substantial differences in exon skipping efficiencies in vitro were observed between oligomers of the same sequence but with different backbone chemistries, with the phosphorodiamidate

morpholino oligomer being the most consistent in inducing exon excision. Similarly, differences in exon excision were observed when normal or exon 2 duplicated cells were transfected, indicating that exon context plays a major role in influencing oligomer induced splice switching. Single exon skipping could be induced in the exon 2 duplicated cells in a dose dependent manner, potentially providing a simpler strategy to restore the reading frame compared to skipping dystrophin exons 2 to 7.

P6: CHARACTERISATION OF A PRIMITIVE BONE MARROW DERIVED MESENCHYMAL STEM CELL SUBPOPULATION SHOWING IMPROVED PROSTATE CANCER TROPISM

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Metastatic cancer remains the main cause of cancer death. Recently, our interest in bone marrow (BM) derived mesenchymal stem cells (BMSC) has led to investigations into why BMSC are attracted to cancer and how to best capitalise BMSC-cancer tropism for the development of novel cell-based therapies for metastatic prostate cancer (PCa). We anticipate a greater understanding of the molecular events governing BMSC tropism for cancer may permit improved therapeutic BMSC targeting to metastatic cancer.

We have isolated a subpopulation of C57BL/6 mouse BM stromal cells showing improved migration toward PCa conditioned media in an *in vitro* transwell migration assay (BD Biosciences) when compared to the pre-sorted cells. These cells were sorted from early passage BM stromal cells following dual colour staining with antibodies against a stem cell marker and a haematopoietic marker. Cells expressing a high level of stem cell marker alone were sorted (FACSVantage, BD Biosciences). This sorted BMSC subpopulation showed a typical BM stromal cell phenotype: stem cell antigen-1^+ [Sca-1^+] $CD44^+$, $CD90^+$, c-kit^+ [$CD117^+$], $CD31^-$, $CD34^-$ and $CD45^-$, while showing enhanced stem cell function (1,2). Specifically, when compared to the pre-sorted cells, the subpopulation showed: enhanced self-renewal; increased colony formation in a fibroblast colony forming unit (CFU-F) assay; increased levels of tri-lineage differentiation into fat, bone and cartilage.

Taken together, these data show we have isolated a phenotypically unremarkable BMSC subpopulation with enhanced stem cell functional properties suggesting an enrichment of primitive, multipotent BMSC. Identification of this primitive BMSC subpopulation offers a unique tool to further explore the molecular events governing BMSC tropism for cancer.

1. Dominici, MD et al., Cytotherapy, 8(4):315-17, 2006.

2. Morikawa S et al., J. Exp. Med., 206:2483-96, 2009.

P7: THE MISNOMER OF ACTIVATING P14ARF-P53 FOR BREAST CANCER THERAPY

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This project challenges the established opinion that gene therapies activating the p53 tumour suppressor pathway through p14ARF and its mimetics provides effective breast cancer (BCa) treatment (1). Tumour suppressor gene p14ARF is frequently lost in BCa and its loss plays a major role in cancer

development. The restoration of p14ARF blocks cancer progression. In contrast, our data show reactivating p14ARF-p53 using gene transfer technology in hormone-dependent BCa provides a double-edged sword where these cells stop dividing but remain viable (2). These latent cells retain the potential to proliferate and seed late-onset BCa.

Using stable isotopic labeling in cell culture (SILAC) we have identified a list of proteins that are differentially regulated post p14ARF-p53 activation in MCF-7 BCa cells. These included anti-apoptotic proteins and, interestingly, the annexin family of proteins. Annexins associated with signal transduction pathways and cell survival were upregulated, however, annexin V, associated with apoptosis, was unchanged. We have validated the differential regulation of the annexin family and associated proteins by Western analysis and real-time quantitative PCR. Using Cytoscape software, the annexin family protein interactome was mapped in MCF-7 cells. To determine whether p14ARF regulates the annexin family in other cancer cells, we expressed p14ARF in an osteosarcoma (U2OS) cell line and found no change in annexin expression.

In conclusion, we showed that activating p14ARF-p53 does not provide an effective treatment for BCa and has the potential to seed latent BCa.

1. Huang et al. (2003) Cancer Res 63: 3646-3653.

2. McGowan EM, et al. (2012) PLoS One 7: e42246.

P8: USE OF CHROMATIN-MODIFYING DRUGS TO CHANGE RETROVIRAL VECTOR INTEGRATION PATTERNS IN HUMAN CD34$^+$ HAEMATOPOIETIC STEM/PROGENITOR CELLS

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Vector-mediated insertional mutagenesis has presented itself as a challenge for the continuing success of gene therapy to treat haematopoietic diseases. Previous studies in haematopoietic cells have shown that the highest frequency of γ-retroviral (γ-RV) vector integration is near high risk genetic loci, like proto-oncogenes. This project aims to evaluate the use of two therapeutically recognised chromatin modifying agents during γ-RV and lentiviral transduction to change the pattern of integration in CD34$^+$ human peripheral blood cells. Human CD34$^+$ cells were isolated from frozen stores of mobilised human peripheral blood. Cells underwent 48 hour pre-stimulation in a cytokine cocktail (300 ng/mL SCF, 300 ng/mL FLT-3L, 100 ng/mL TPO, 20 ng/mL IL3) prior to transduction, with the addition of 5-Aza-2′-deoxycytidine (10^{-6}M) for the last 16 hours. Cells subsequently underwent three rounds of transduction with a γ-RV vector, encoding MGMT (cumulative MOI 4.5) with the previous cytokine cocktail and the addition of Trichostatin A (5 ng/mL). CD34 positivity and MGMT expression were established by flow cytometry. Both the retention of CD34 positivity and the efficiency of transduction were affected by the drug treatment. Drug treated cells were found to be 49.9% CD34$^+$ (control 35.9% n=3) from a starting positivity of 92.9%, while the MGMT expression was 3.53% (control 20.63% n=3). This indicates that the drug regime had a physiological effect on the cells. Vector/gDNA junction fragment libraries are currently being constructed for next generation sequencing to assess the effects of drug-induced chromatin remodelling on γ-RV integration patterns and signatures.

P9: DEVELOPMENT OF rAAV VECTORS FOR THE TREATMENT OF

LEUKODYSTROPHIES

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Acute or chronic demyelination underlies the pathology of leukodystrophies. Inherited myelin diseases are typically caused by single gene mutations altering function or viability of oligodendrocytes or astroglia. Recombinant adeno-associated viral (rAAV) vectors have proven to be safe and versatile tools for gene transfer to the central nervous system. Promoter selectivity is sufficient to target transgene expression to glia in adult rodents. However, this approach has not been exploited with regard to efficacy studies or vector delivery at early postnatal stages. Here we examined AAV vector tropism and spread by driving GFP expression with oligodendrocyte-derived myelin basic protein (MBP-), myelin associated glycoprotein (MAG-) or the astrocyte derived glial fibrillary acidic protein (GFAP-) promoters at three weeks post intrastriatal injection into neonatal, juvenile or adult mice. Our results demonstrate that depending on the promoter and developmental stage of the animal, transgene expressing cell types targeted largely varied. Remarkable astrocyte specificity was achieved with the GFAP promoter independent of the developmental stage. In contrast, negligible transgene expression was observed in oligodendrocytes after neonatal delivery of AAV-MBP-GFP. Oligodendroglial specificity using this promoter was high upon delivery at postnatal day p10 or in adults. The vector spread was comparable for delivery at early stages but markedly reduced upon adult delivery. *In vivo* targeted transgene expression depends on promoter and developmental characteristics at intervention and only somewhat on the serotype. Timing of interventions aiming at gene delivery to glia requires in depth knowledge on the efficacy profile of the selected cell-type specific promoter.

P10: PEPTIDE NUCLEIC ACIDS FOR SELECTIVE INHIBITION OF CELLS CONTAINING THE *MDX* DYSTROPHIN LOCUS

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Introduction: Peptide Nucleic Acids (PNAs) are oligonucleotide analogues composed of nucleobase derivatives attached to a neutral polyamide backbone. PNAs follow Watson-Crick base pairing principles due to their nucleobase components and exhibit a much higher annealing affinity for complementary DNA strands than oligonucleotides. This strong affinity for complementary nucleotide strands results in strong strand invasion and targeting making PNAs extremely efficient as gene targeting molecules. This aspect of PNA chemistry and molecular biology is enhanced from an application standpoint by PNAs' chemical stability and resistance to intracellular nucleases and DNA processing enzymes. Utilising these aspects of PNA chemistry, we have designed PNA molecules to specifically inhibit the growth of myoblasts that contain the *mdx* dystrophin gene mutation compared to myoblasts that do not, by their 100% homology (9-mer PNA) with the *mdx* mutation compared with only 89% homology with the *wt* (non-*mdx*) nucleotide. *mdx* and *wt* myoblasts were electroporated with a fluorescently labelled 9-mer PNA and positively selected by FACS. The proliferative behaviour of these cells was then assessed using the PicoGreen DNA quantification assay. Our results show that the PNA inhibits the growth of *mdx* myoblasts to a significantly greater extent than *wt* myoblasts. This is evident up to 72 hours post electroporation. This work provides a means by which cells containing corrected gene loci can be

expanded from a cell population consisting of cells with mutant (*mdx*) and non-mutant (*wt*) loci.

P11: EXCEPTIONS TO EXCEPTIONS IN THE DUCHENNE:BECKER DYSTROPHIN READING FRAME

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The majority of mutations in the dystrophin gene lead to either a severe condition, Duchenne muscular dystrophy, or a milder allelic condition Becker muscular dystrophy. The dystrophin reading frame generally correlates disease severity with the genotype, but there are notable exceptions in about 10% of cases. Dystrophin protein truncating mutations have been described that manifest with only mild symptoms, while in-frame deletions may present with an unexpectedly severe phenotype. One such exception to the reading frame rule is the deletion of dystrophin exon 5, an in-frame deletion that has been reported to manifests a Duchenne muscular dystrophy. The severe phenotype in two unrelated dystrophin exon 5 deletion patients was attributed to the deletion of both exons 5 and 6 from the mature mRNA, with disruption of the reading frame. Here, we describe a patient with a genomic deletion of dystrophin exon 5 who presents with relatively mild symptoms. Unlike the cases reported above, the mRNA transcribed from this mutation retained exon 6. Deletion breakpoint mapping indicated that part of introns 4 and 5 were also deleted from the genomic DNA and the deletion was larger than other deletions reported around this exon. Application of a splice switching oligomer targeting dystrophin exon 6 removed that exon from the mature mRNA at efficiencies similar to that observed following testing in normal myogenic cells, indicating this particular loss of dystrophin exon 5 did not compromise exon 6 recognition. It is evident that not all dystrophin genomic deletions are processed in a similar manner.

P12: GENE REPAIR OF THE *ORNITHINE TRANSCARBAMYLASE* (*OTC*) LOCUS IN *SPFASH* MICE USING ADENO-ASSOCIATED VIRUS (AAV) INDUCED HOMOLOGOUS RECOMBINATION

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Repair of mutant loci via AAV-mediated homologous recombination (HR) demonstrates 1000-fold greater efficiencies over alternative approaches such as plasmid DNA and adenovirus. Using the OTC-deficient spf^{ash} mouse model, this project aims to optimise AAV-HR repair in cells possessing therapeutic potential (inducible pluripotent stem cells and liver progenitor cells (LPC)) for treatment of monogenic liver disease. An AAV vector was constructed to target and repair the *Otc* locus at exon 4 and introduce downstream *Otc* exons 5-10 and a polyadenylation signal. A *neomycin phosphotransferase* expression cassette was also included to permit G418-selection of transduced cells. Functionality of the construct (OTCe4-neo) was validated in male neonatal mice injected with 2.2×10^{11} vg of the vector packaged in hepatotropic serotype 8 capsids. Livers were harvested six weeks later and shown by histochemical analysis to

contain clusters of OTC positive cells, consistent with the notion that locus repair had occurred in hepatocytes prior to cellular division(s). OTCe4-neo was packaged into capsid serotype 1, which possessed the best transduction efficiencies of serotypes 1-10 in murine LPC reporter assays (followed by AAV serotypes 2 and 6). LPC were then transduced with AAV1-OTCe4-neo at MOI = 60,000 or 100,000 vg/cell. Signals specific for targeted recombination were detected by nested PCR in pooled populations of G418-resistant cells. Further studies are needed to (i) determine the frequency of targeted recombination, (ii) repeat the findings in *spf*^{ash} LPC and (iii) test restoration of OTC expression after *in vitro* differentiation or *in vivo* engraftment.

P13: HSV-1 AMPLICON MEDIATED DELIVERY OF THE HUMAN β-GLOBIN LOCUS

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Lentiviral (LV) vectors are currently the most common delivery mechanism for gene therapy, due to their capacity to carry moderate quantities of foreign DNA (~8 kb), infect dividing and quiescent cells, and integrate into the host cell genome. A clinical trial of a LV vector carrying a β-globin transgene to treat β-thalassemia demonstrated the utility of this approach, while also exposing the limitations of such vectors. LV delivery of the β-globin gene resulted in increased haemoglobin (Hb) levels, but did not achieve Hb concentrations in the healthy range. This result may be related to the limited capacity of LV; truncation of regulatory elements, necessary to create a transgene within the size limits of the vector, is likely to result in reduced expression relative to the wild type gene. This will particularly be an issue where wild type expression levels are very high, as is the case for β-globin.

To address this problem, we are adapting the Herpes Simplex Virus-1 (HSV-1) amplicon system for delivery of a genomic β-globin transgene. The HSV-1 virus has a genome of 152kb, and can be adapted to carry up to 150kb of foreign DNA. We have created a BAC containing the complete β-globin locus, retrofitted with sequences required for HSV-1 packaging via cre-lox recombination. This construct has been packaged into infectious HSV-1 particles, capable of delivering the complete β-globin locus. Ongoing work is aimed at transduction of erythroid cells with HSV-1 vectors, and developing methods for integration of the genomic transgene into the host genome.

P14: RESTORATION OF CORRECT SPLICING OF β$^{IVS2-654}$ THALASSEMIC PRE-MRNA BY MODIFIED ANTISENSE U7 SNRNA LENTIVIRAL VECTOR

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One of the most common mutations causing β-thalassemia in Thailand and South East Asia is a C-to-T mutation at nucleotide 654 of human β-globin intron 2 (IVS2-654). This mutation creates aberrant β-globin pre-mRNA splicing and prevents full-length β-globin chain synthesis. It has been shown that masking of aberrant splice sites or elements involved in aberrant splicing with synthetic antisense oligonucleotides can switch the aberrant splicing pattern to the therapeutic correct splicing and restore β-globin expression. To achieve long-term masking of aberrant splice sites or element involved in aberrant splicing, a modified U7 small nuclear RNA (U7 snRNA) lentiviral vector was used to delivered antisense sequence for stable expression in target cells. In this study, the

lentivirus-delivered U7.BP+623 snRNA which targets a cryptic exonic splicing enhancer and a cryptic branch point of the aberrant splicing was evaluated in a HeLa cell model system expressing the aberrantly spliced enhanced green fluorescent (EGFP-654) pre-mRNAs and erythroid progenitor cells from βIVS2-654 thalassemic patients. The U7.BP+623 snRNA lentiviral vector was properly expressed and was found to exhibits a significant increase in corrected aberrant splicing in the HeLa EGFP-654 cell line for at least 3 months as shown by up-regulation of EGFP expression. Importantly, the correctly spliced β-globin mRNA was achieved in IVS2-654/HbE thalassemic erythroid progenitor cells transduced with U7.BP+623 snRNA lentiviral vectors, leading to upregulation of hemoglobin A. Our study demonstrates a proof of principle for splice-switching therapy for β-thalassemia.

P15: SILENCING A DISEASE MODIFYING MUTATION FOR CYSTIC FIBROSIS USING ANTISENSE OLIGONUCLEOTIDES

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Cystic fibrosis, the most common genetic disorder, affects 1 in 2500 Caucasians. There are 800 known mutations to the Cystic Fibrosis Transmembrane Conductance Regulator Gene (*CFTR*), of which, 14% affect mRNA splicing. Mutations to *CFTR* interfere with function of the CFTR chloride channel protein, causing atypical chloride transport across lung epithelium. One common *CFTR* mutation known as c.3717+12191C>T, causes an 84 base pseudoexon within intron 22. This transcript is unstable and quickly degraded. However, low levels of normal *CFTR* are sufficient for essential airway functioning resulting in a mild CF phenotype.

Additional mutations on *CFTR* can further enhance the disease phenotype of patients carrying this mutation. In particular, a polymorphism in the number of Thymine bases in the pyrimidine tract of intron 9, can weaken recognition of the exon 10 splice site, causing frequent skipping of exon 10 from the transcript. We have obtained a patient cell line homozygous for c.3717+12191C>T as well as for the 7T allele. This causes approximately 50% of transcripts to skip exon 10, resulting in a more severe CF phenotype. Antisense Oligonucleotides can specifically manipulate splicing by blocking or enhancing splice sites. We have designed AOs for pseudoexon removal as well as AOs to induce exon 10 inclusion back into the transcript. We speculate that AOs used to treat both mutations in combination will increase levels of normal CFTR protein that would improve disease phenotype in CF patients affected by this disease modifying polymorphism.

P16: EVALUATION OF FOETAL HAEMOGLOBIN REGULATORS THROUGH RNA INTERFERENCE

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The human β-globin locus consists of embryonic, foetal and adult globin genes, which are sequentially expressed during ontogeny. Individuals with β-globin gene defects result in adult haemoglobin disorders such as β-thalassaemia and sickle cell disease. The clinical symptoms of these disorders are significantly ameliorated by the persistent expression of foetal γ-globin after birth. Recent genome-wide association studies of patients harbouring hereditary persistence of foetal haemoglobin have identified three quantitative trait loci including the *BCL11A* locus and the *HBS1L-MYB* intergenic region. A greater understanding of the regulatory elements that control γ-globin expression could help to identify new therapeutic targets

for patients with β-haemoglobinopathies. Here, we report the knockdown of known γ-globin silencing factors *Myb, Bcl11a* and *Dnmt1* by RNAi in a robust genomic reporter assay system. Knockdown was performed in murine erythroleukaemic cells containing a 183 kb intact human β-globin locus where the γ-globin gene and β-globin genes are replaced by DsRed and eGFP fluorescent-reporters, respectively. Following RNAi, key regulators governing γ-globin regulation were measured by RT-qPCR. We show significant induction of γ-globin following knockdown of *Myb*, *Bcl11a* and *Dnmt1*. Furthermore we show multiple regulatory pathways of *Myb* in the silencing of γ-globin. Our results demonstrate the utility of the fluorescence-based cellular assay for investigating regulatory elements that control γ-globin expression in an adult erythroid environment. Identifying key regulators could pave the way for new therapeutic treatments of β-haemoglobinopathies.

P17: UPREGULATION OF MIR-210 IN β-THALASSEMIA/HBE ERYTHROID

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Thalassemia is a congenital hemolytic disorder that results in defective globin chain production. This situation causes excess unbound α-globins to precipitate in red blood cell precursors leading to premature death in bone marrow that causes ineffective erythropoiesis and severe anemia. miR-210, a hypoxia-induced miRNAs, has been shown to regulate globin gene expression. This study investigates miR-210 expression in red blood cells of thalassemia patients. Increased level of miR-210 expression was observed in β-thalassemia/HbE patients compared with that of normal individuals ($p < 0.05$). The miR-210 expression levels were inversely correlated with hemoglobin level ($r^2 = -0.7054$, $p < 0.05$) and percentage of hematocrit ($r^2 = -0.6017$, $p < 0.05$). Expression of miR-210 during erythropoiesis in β-thalassemia/HbE patients were examined. We found that miR-210 expression level was increased in thalassemia patients when compared with that of normal individuals and was highest at basophilic and polychromatic normoblast stages of erythroid differentiation. Moreover, we investigated the expression of miR-210 in β-thalassemic mice. We found that miR-210 expression in β-thalassemic mice was also elevated during erythropoiesis when compared to wild type mice. Upregulation of miR-210 was also observed in basophilic and polychromatic normoblast stages in β-thalassemic mice. This study indicates that our β-thalassemic mice represent a suitable model system to study hypoxia-induced miRNA.

P18: USE OF LENTIVIRUS TRANSDUCTION TO INDUCE LONG-TERM SYNTHESIS AND LOCALISED SECRETION OF AN IMMUNOSUPPRESSIVE PEPTIDE

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Autoimmune diseases are caused by immune responses that are directed against self-antigens within the body of the person leading to inflammation and eventual tissue damage. Examples of such destructive autoimmune diseases include Type 1 diabetes (T1D),

rheumatoid arthritis, multiple sclerosis, and psoriasis. These diseases are chronic and debilitating, requiring lifelong care, monitoring and significant treatment. Despite the varied clinical presentation of these diseases one shared pathogenic mechanism common to most is that they require the presence of self-reactive $CD4^+$ T-cells to induce tissue damage. The aim of this project was to induce the secretion of a novel peptide specific T-cell inhibitor (Core Peptide, CP) in antigen presenting cells such as dendritic cells and target cells of autoimmune reaction, such as islet cells in T1D, as a novel means of immunosuppression using gene therapy. Various DNA constructs encoding for CP were designed, with different signal peptides and some encoding for GFP, and subcloned into lentivirus plasmids. Western blotting was used to detect the intracellular production of CP in transfected/transduced cells. We also utilised a functional assay (mixed lymphocyte reaction) to demonstrate the suppression of co-cultured T-cells inferring that there was production of CP by transduced cells. These results indicated that cells may be induced to secrete a local immunosuppressive peptide with the use of a lentivirus encoding for CP. This genetic approach may provide a new method to treat autoimmune diseases by preventing T-cell mediated tissue destruction.

P19: GENE THERAPY FOR XLA: AN ANIMAL MODEL USING HUMAN BRUTONS TYROSINE KINASE (BTK)-NEGATIVE CELLS

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X-Linked Agammaglobulinaemia (XLA) is a primary immune deficiency (PID) resulting in an absence of B cells and antibody production due to mutations in the Btk gene. Treatment requires lifelong immunoglobulin replacement with the risk of significant morbidity and reduced quality of life scores. Gene therapy for PIDs has evolved significantly over the last few years with human trials being successful. XLA is a good candidate for gene therapy with a known molecular defect; the gene- modified cells have a probable selective advantage; and therapy can be done without the need to withdraw treatment of immunoglobulin. The aim is to achieve efficient transduction and genetic repair of Btk deficient human $CD34^+$ cells using a lentiviral construct and to demonstrate resultant functional reconstitution of the B cell compartment in a humanised mouse model (NSG). We have developed a lentiviral vector encoding the human Btk cDNA driven by the ubiquitous Elongation Factor 1-alpha promoter and have reconstituted the NSG mouse model with Btk positive wildtype human $CD34^+$ cells using Busulphan as the conditioning agent. Full engraftment was demonstrated in female mice at doses ranging from 0.35-5 $x10e^6$ $CD34^+$ cells; interestingly Graft versus host disease (GVHD) in liver and skin was observed in all groups with the higher doses developing GVHD earlier. We have recently obtained our first XLA (Btk negative) patient $CD34^+$ cells, transduced with our viral vector and transplanted into the NSG mouse; the results are awaited.