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7th AUSTRALASIAN GENE THERAPY SOCIETY MEETING

Date

May 4 – May 6, 2011

Venue

Bio21 Institute
University of Melbourne
Parkville, Victoria, Australia

The Australasian Gene Therapy Society (AGTS) held its Seventh Biennial Meeting between 4th May and 6th May 2011. 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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Broadvector Limited
North Ryde, NSW, Australia

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Gene Therapy Research Unit
Children's Medical Research Institute
Westmead, NSW, Australia

Paul Gregorevic
Baker IDI
Laboratory for Muscle Research and Therapeutics Development
Melbourne, VIC, Australia

Rose Martiniello-Wilks
School of Medical and Molecular Biosciences,
University of Technology,
Sydney, NSW, Australia

Ann Simpson
School of Medical and Molecular Biosciences,
University of Technology,
Sydney, NSW, Australia

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KEYNOTE PRESENTATIONS

KEYNOTE 1: SMALL RNA THERAPEUTICS FOR THE TREATMENT OF HIV

John J. Rossi[1], Jiehua Zhou[1], John A. Zaia[1], David DiGiusto[1], Ling Peng[2], Preston Neff[3] and Ramesh Akkina[3], [1]Beckman Research Institute of the City of Hope, Duarte, CA.;[2]Marseilles University, Marseilles, France; [3]Colorado State University, Fort Collins, CO.

A goal of our research is the application of small RNA based therapeutics for the treatment of HIV-1 infection. To accomplish this we are exploring two alternative molecular approaches. The first is a gene therapy application of combinations of small anti-HIV inhibitory RNAs. We have introduced these via a lentiviral vector into hematopoietic progenitor cells and carried out autologous stem cell transplants in AIDS/lymphoma patients. Results to date of this four patient trial will be presented. As a second approach a novel dual inhibitory function anti-gp120 aptamer-siRNA delivery system for HIV-1 therapy is being developed

in which both the aptamer and the siRNA portions have potent anti-HIV activities. The envelope glycoprotein is expressed on the surface of HIV-1 infected cells, allowing binding and internalization of the aptamer-siRNA chimeric molecules. The Dicer-substrate siRNA delivered by the aptamers is functionally processed by Dicer, resulting in specific inhibition of HIV-1 replication and infectivity in cultured CEM T-cells and primary blood mononuclear cells.

A third approach uses a PAMAM G5 dendrimer for non targeted delivery of Dicer substrate small interfering RNAs in human CD4+ T-lymphocytes. Our results show efficient nanoparticle formation of G5 dendrimers with our siRNAs, effective delivery to the target cells and the release of siRNAs that are processed by Dicer into functional 21-22mer siRNAs which are incorporated into the RNA induced silencing complex (RISC) and guide sequence specific degradation of the target transcripts.

For both the aptamer-siRNA and dendrimer-siRNA delivery systems their effectiveness was examined in a humanized SCID mouse model that is reconstituted with human hematopoietic cells that are fully capable of infection by HIV. A group of humanized mice were treated with virus until they became viremic. Subsequently the animals were treated with either the aptamer-siRNA or the dendrimer-siRNA combinations by giving three to five weekly tail vein injections. We show that the in vivo applications of both the aptamer-siRNA and the dendrimer siRNAs resulted in three to six logs of inhibition of viral replication, siRNA mediated down regulation of the targeted mRNAs and protection of T-lymphocytes from HIV mediated depletion. These results represent the first such small RNA applications for the successful treatment of HIV-1 infection, and either approach could potentially be used in HIV-1 eradication strategies.

KEYNOTE 2: PATIENT-DERIVED STEM CELL MODELS OF BRAIN DISEASES

Alan Mackay-Sim, National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Queensland.

We are using patient-derived stem cells to investigate monogenic brain diseases and those of complex genetic and environmental origin. We compare cells from multiple patients and controls carrying the full complement of genetic variation to identify disease-associated dysregulation of gene expression and cell functions. Patient-derived stem cells are obtained from biopsies of olfactory mucosa which is regenerated throughout life from a multipotent neural stem cell. Our adult stem cell bank currently comprises olfactory stem cells from 200 people including healthy controls and those with various brain diseases. Our initial experiments investigated schizophrenia, a neurodevelopmental psychiatric disorder, and sporadic Parkinson's disease, a neurodegenerative disease. Using multiple patient and control cell lines we demonstrated significant disease-specific alterations in gene and protein expression, and cell signaling pathways involved in neurodevelopment in schizophrenia and in mitochondrial function and xenobiotic metabolism in Parkinson's disease. Subsequently we have identified specific molecules involved in dysregulated cell cycle and cell migration in schizophrenia and in oxidative stress and responses to environmental toxins in Parkinson's disease. Using the same approach in Hereditary Spastic Paraplegia we demonstrated widespread dysregulation of gene expression and significantly altered distributions of intracellular organelles. In MELAS, a mitochondrial tRNA mutation disorder, mitochondrial function was correlated with tRNA mutational load. These results show that these adult neural stem cells demonstrate significant disease-specific alterations in cell

signaling pathways that are useful for understanding disease aetiology, drug discovery and, in the case of monogenic diseases, as models for gene therapy.

KEYNOTE 3: GENERATION OF KNOCKOUT RATS USING ZINC-FINGER NUCLEASES

Tomoji Mashimo, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Although the laboratory rat has been widely used as animal models across many fields in biomedical sciences, the inability to utilize germline-competent embryonic stem (ES) cells was a major drawback for research activities that aimed to elucidate gene functions. Recently, Zinc finger nucleases (ZFNs) have been used successfully to create site-specific DNA double-strand breaks, and thereby stimulate targeted gene mutations in a wide variety of organisms including plants, xenopus, drosophila, zebrafish, and rats. Here we demonstrate ZFN-stimulated gene-targeting at an endogenous rat gene, for which human and mouse mutations are known to cause a severe combined immunodeficiency (SCID). Co-injection of mRNAs encoding the custom-designed ZFNs into pronucleus of fertilized oocytes yielded 25% gene-modified offspring, including a wide variety of deletion or insertion mutations. ZFN-modified founders faithfully transmit these genetic changes to the next generation with the SCID phenotypes. The high frequency of gene-targeting and the rapid creation of gene knockouts indicate that ZFN technology can provide a new strategy in rats for creating animal models of human diseases.

KEYNOTE 4: RNA AT THE EPICENTRE OF HUMAN DEVELOPMENT

John S. Mattick, Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia.

It appears that the genetic programming of humans and other complex organisms has been misunderstood for the past 50 years, because of the assumption that most genetic information is transacted by proteins, leading to the derived assumptions that most intronic, intergenic and retrotransposed sequences in these genomes are non-functional. Surprisingly, however, the human genome appears to contain only about 20,000 protein-coding genes, similar in number and with largely orthologous functions as those in nematodes that have only 1,000 cells. On the other hand, the extent of non-protein-coding DNA increases with increasing complexity, reaching 98.8% in humans. The majority of these sequences are dynamically transcribed, mainly into long and short non-protein-coding RNAs, of which there are tens if not hundreds of thousands that show specific expression patterns and subcellular locations. These include not only miRNAs, but also new classes of small RNAs derived from small nucleolar RNAs, transcription start sites and splice sites, and new classes of long ncRNAs derived from developmental enhancers and post-transcriptional processing of mRNAs. The emerging evidence indicates that these RNAs form a massive hidden network of regulatory information to control gene expression at various levels, including the site-specificity of the chromatin-modifying complexes that underpin developmental trajectories and cognitive function, and that these regulatory RNAs are dysregulated in cancer and other complex diseases. While it is relatively easy to revert cells to a pluripotent state, the amount of information required tocontrol the forward processes of 4-dimensional differentiation and development is enormous. It is also becoming evident that animals, particularly primates, have superimposed plasticity on these RNA regulatory systems by RNA editing, and that this is the molecular basis of the environment-epigenome interactions that underpin brain function and (in all likelihood) complex diseases. Retrotransposons also appear to

contribute to genomic plasticity and somatic mosaicism, especially in the brain. Thus, it appears that most assumptions about the nature and structure of regulatory information have been incorrect, and that what was dismissed as 'junk' because it was not understood will hold the key to understanding animal evolution, development, phenotypic diversity and cognition, with far-reaching implications for science, medicine and biotechnology.

KEYNOTE 5: SUPPRESSION OF DUX4 OR DUX4C EXPRESSION BY ANTISENSE STRATEGIES IN A THERAPEUTIC APPROACH FOR FSHD

C. Vanderplanck[1], E. Ansseau[1], S. Charron[1], A. Tassin[1], A. Turki[2], D. Laoudj-Chenivesse[2], S.D. Wilton[3], F. Coppée[1] and A. Belayew[1], [1]Lab. Molecular Biology, University of Mons, Belgium, [2]INSERM U1046, University of Montpellier, France, [3]Molecular Genetic Therapy Group, University of Western Australia, Nedlands, WA, Australia.

Facioscapulohumeral muscular dystrophy (FSHD) is genetically linked to deletions in 4q35 within an array of D4Z4 repeated elements in which we mapped the double homeobox 4 (DUX4) gene. We found stable DUX4 mRNAs only derived from the distal D4Z4 unit and unexpectedly extended to a polyadenylation signal within the flanking pLAM region. DUX4 encodes a transcription factor expressed in FSHD but not control primary myoblasts. DUX4 initiates a large transcription deregulation cascade leading a.o. to muscle atrophy and oxidative stress, which are FSHD key features. We also characterized the homologous DUX4c gene in 4q35 that is expressed in healthy myoblasts and induced in FSHD: its over-expression triggers myoblast proliferation.

We show here that DUX4 expression in human myoblast cultures induces E3 ubiquitin ligases associated with muscle atrophy and the formation of atrophic myotubes. In contrast DUX4c induces β-catenin stabilization and formation of disorganised myotubes with large clusters of myonuclei. Interestingly, FSHD primary myotubes present different proportions of these atrophic/disorganized phenotypes.

We reasoned that inhibition of DUX4 or DUX4c expression should prevent the transcription deregulation cascade and restore the myotube phenotype. We either induced mRNA destruction by RNA interference (siRNAs) or affected splicing by specific antisense oligomers in human myoblast cultures. Decreased DUX4 or DUX4c protein was confirmed by immunodetection on western blots and the biomarker and myotube phenotypes changed as expected. These strategies could lead to therapeutic approaches for FSHD.

We acknowledge funding from the AFM (France). CV and SC were FRIA graduate fellows, AT and EA post-doctoral FNRS associates (Belgium).

KEYNOTE 6: SYNTHETIC AAV VECTORS AS TOOLS FOR SAFE shRNA EXPRESSION AND TARGETED MIRNA REGULATION

Dirk Grimm, *Heidelberg University, Cluster of Excellence CellNetworks, Dept. of Infectious Diseases, Virology, Heidelberg, Germany.*

The juxtaposition of viral gene transfer vector and RNAi gene silencing technologies certainly represents one of the most potent and versatile currently pursued avenues for therapeutic intervention with a large variety of human diseases. One particularly promising viral candidate for RNAi vector development is the Adeno-associated virus (AAV) family due to its unusual combination of clinically beneficial inherent properties, from the apathogenicity of the wildtype virus and vectors derived thereof, to the availability of a

plethora of naturally occuring AAV variants with distinct features. In an effort to further expand the genuine repertoire of viral capsids and to ultimately realize the concept of personalized biomedicine, we and others have recently begun to molecularly evolve massive diverse libraries of synthetic AAV particles that can now be screened for individual vectors with user-defined properties. In parallel, we and other labs have continuously improved the design of our AAV vector cassettes for the expression of therapeutic short hairpin RNAs (shRNAs) against pathogenic viruses and further clinically relevant targets, including ingenious novel strategies to particularly enhance the safety of these recombinant tools. Last but not least, we and others are concurrently engineering our vectors as precise modulators of disease-associated miRNAs or are *vice versa* exploiting cellular miRNAs to increase vector target specificity. In this presentation, I will provide a comprehensive insight into these three closely related exciting ongoing developments in the field of RNAi therapies and will discuss the critical looming steps to realize and foster the clinical translation of these promising new principles.

KEYNOTE 7: GENE TARGETING WITH ADENO-ASSOCIATED VIRUS VECTORS

David W. Russell, University of Washington, Seattle, WA, USA

AAV vectors efficiently recombine with homologous chromosomal sequences in mammalian cells. Up to 1% of the entire cell population exposed to vector particles undergoes gene targeting under optimal conditions. Insertions, deletions and substitutions can all be introduced with high fidelity. The technique works well in many cell types, including transformed cell lines, stem cells, and even in vivo in hepatocytes. In this presentation I will review our recent gene targeting work with human pluripotent stem cells. We have used AAV vectors to eliminate disease-causing genes such as those responsible for osteogenesis imperfecta and to manipulate chromosomes, including trisomy removal from Down syndrome cells.

The Greg Johnson Memorial Oration

PATHWAYS REGULATING BLOOD CELL PRODUCTION

Douglas Hilton & many generous and valued colleagues in the Divisions of Molecular Medicine, Cancer & Haematology, Molecular Immunology and Bioinformatics, The Walter and Eliza Hall Institute of Medical Research and the Department of Medical Biology, University of Melbourne.

While an undergraduate and PhD student in the Cancer Research Unit at WEHI in the mid to late 1980s, I was privileged to work alongside Greg Johnson. Greg was a generous and warm teacher and was a wonderful example that great science could go hand-in-hand with having good fun. I have a copy of Greg's PhD thesis "Hepatic Haemopoiesis: A Developmental Study" on my bookshelf and refer to it regularly. I consider it a tremendous honour to be the 2011 Greg Johnson Orator.

My current research takes genetic, genomic and bioinformatic approaches to identify gene networks important in regulating haemopoiesis; the production of nine lineages of blood cells from multipotential, self-renewing stem cells. In this presentation I will discuss our most recent published and unpublished experiments that focus on stem cells and commitment to the megakaryocyte lineage.

INVITED PRESENTATIONS

IS1: TARGETING DRUG RESISTANCE IN LUNG CANCER

Maria Kavallaris, Children's Cancer Institute Australia, Lowy Cancer Research Centre, University of New South Wales, Randwick, NSW, 2031, Australia.

Lung cancer is the most common cancer and despite advances in treatments, overall 5 year survival rates for advanced disease are dismal. ßIII-tubulin (encoded by the TUBB3 gene) is a neuronal-specific cytoskeletal protein that is associated with aggressive tumours and drug resistance in a range of cancer types including non-small cell lung cancer (NSCLC), ovarian, and breast cancers (1). Silencing the microtubule protein βIII-tubulin using siRNA sensitises NSCLC cells to tubulin-binding agents such as paclitaxel and the *vinca* alkaloids (2). Moreover, silencing of βIII-tubulin also increases sensitivity to DNA damaging agents such as cisplatin, etoposide and daunorubicin (2). NSCLC cells stably expressing βIII-tubulin short hairpin RNA displayed increased chemotherapy sensitivity and reduced growth when compared with control clones. In concordance with these results, stable suppression of βIII-tubulin reduced the incidence and significantly delayed the growth of tumours in mice relative to controls. Our findings have demonstrated that βIII-tubulin mediates not only drug sensitivity but also the incidence and progression of lung cancer (3). βIII-Tubulin has emerged as a promising gene therapy target for the treatment of advanced NSCLC.

1. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer. 2010;10:194-204.

2. Gan PP, Pasquier E, Kavallaris M. Class III beta-tubulin mediates sensitivity to chemotherapeutic drugs in non small cell lung cancer. Cancer Res. 2007;67:9356-63.

3. McCarroll JA, Gan PP, Liu M, Kavallaris M. βIII-Tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. Cancer Res. 2010;70:4995-5003.

IS2: USE OF AUTOLOGOUS PERIPHERAL BLOOD T LYMPHOCYTES TRANSDUCED WITH AN ANTI LEWISY CHIMERIC RECEPTOR GENE IN PATIENTS WITH LEWISY POSITIVE MULTIPLE MYELOMA, ACUTE MYELOID LEUKAEMIA OR HIGH-RISK MYELODYSPLASTIC SYNDROME

Amit Khot[1], Dominic Wall[1,2], Michael Kershaw[1], Jennifer Westwood[1], Joseph Trapani[1], Mark Smyth[1], Phillip Darcy[1], Andrew Scott[1], Dirk Honemann[1], Lucy Kravets[1], Paul Neeson[1], Rodney Hicks[1], Stefan Peinert[1], H Miles Prince[1,2], [1]Peter Maccallum Cancer Centre, Melbourne, Victoria, Australia, [2]University of Melbourne, Victoria, Australia.

High-risk multiple myeloma (MM), acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) have a dismal prognosis with available treatments. Immunomodulation offers a novel approach. We have shown that Lewis^Y, a carbohydrate antigen is strongly expressed in 27% of MM patients and 46% of AML patients, and engineered a chimeric receptor consisting of extracellular humanized scFv recognizing the Le^Y Ag, linked to an extracellular CD8 hinge region, a transmembrane and cytoplasmic CD28 signaling domain, and zeta signaling chain. The humanized anti- Le^Y scFv-CD28-ζ receptor has been cloned into the pSAMEN retroviral vector and transfected into the packaging line PG13. To ensure that the retrovirus is replication deficient, structural genes are removed and replaced with genes of interest, i.e. the gene for the chimeric T-cell receptor.

Pre-clinical data:

- The construct can be successfully introduced into human T-cells

- Transduced anti-Le^Y T-cells (ALTs) proliferate in response to chimeric receptor ligation.

- LeY-expressing tumour cells are lysed by ALTs.

- Systemically delivered cells inhibit tumour growth in mice.

- ALTs are active in a NOD-SCID MM mouse model

- ALTs are not pathogenic in a BALB/c mouse model.

This phase I study to evaluate the safety and tolerability of autologous ALTs has recruited 4/6 patients; adequate numbers of T-cells have been harvested and 3 patients have been infused successfully; the products satisfied GMP release conditions and target cell numbers were achieved during cell expansion. A safety review has not found any adverse events necessitating study termination. This appears to be a feasible strategy to deliver targeted T-cells.

IS3: GENE THERAPY FOR HEART FAILURE

David Kaye, Heart Failure Research Group, Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

Congestive heart failure (HF) is a leading cause of hospitalization, disability and death. Despite significant advances in pharmacotherapy, HF remains a progressive disorder with an unacceptably high mortality rate. While the molecular and cellular basis of the impairment of myocardial contractility has been determined in detail, current pharmacological therapies for heart failure only modify these abnormalities indirectly, by interfering with neurohormonal control of the heart. Accordingly, there has been growing interest in applying gene therapy based interventions in HF. In particular, studies directed at the excitation-contraction pathway of the heart have identified genes (particularly phospholamban and the sarcoplasmic reticulum Ca^{2+}-ATPase -SERCA) that can be manipulated to improve contractility. Translation into clinical practice requires the selection of appropriate gene targets together with the successful integration of safe, minimally invasive delivery methods that provide homogeneous delivery to the heart. We have developed a novel clinically-relevant, percutaneous system for the delivery of viral vectors to the myocardium, based upon an antegrade coronary arterial delivery system combined with recirculation of the coronary venous effluent via a membrane-pump oxygenator circuit. In studies performed to date, we have shown that gene therapy improves cardiac performance in a large animal model, providing support for recent clinical trials of AAV-SERCA gene therapy for HF.

IS4: RNAI THERAPEUTICS SCREENING FOR GENE THERAPY

York Zhu[1,3], Peter French[2], Wei -Peng[1] , Yi-Xiang Lu[1] , Tie-Jun Li[1], Yun-Cheng Sun[1], Xiao-Jun Tang[1] , Li Shan[1], Jay Chen[1] and Jinkang Wang[1], [1]Biomics Biotechnologies Co., Ltd. China (www.biomics.cn), [2]Benitec Limited (ASX:BLT), [3]China Pharmaceutical University, China.

RNA interference (RNAi) is a fundamental cellular mechanism for silencing gene expression and has become a powerful and widely used platform for developing therapeutics of viral infections, cancer, vascular-and other diseases. RNAi based gene knock-down is induced by short double-stranded RNAs also known as small interfering RNAs (siRNAs). An Entire siRNA Target (EsT) Library based on high-throughput screening of RNAi therapeutics has been successfully established in our laboratory. In applying this technology, we have screened a group of RNAi therapeutics for HBV, cancers and other diseases. In this report, we will describe the technical

(technology?) platforms, from in-vitro assay and in-vivo validation as well as delivery systems for these RNAi therapeutics to clinical applications.

IS5: FINDING TARGETS FOR FSH DYSTROPHY THERAPY

Melanie Ehrlich[1,2], Koji Tsumagari[1], Shao-Chi Chang[1], Michelle Lacey[2,3], Carl Baribault[2, 3], Sridar V. Chittur[4], Janet Sowden[5], Rabi Tawil[5], Katherine E.Varley[6], Jason Gertz[6], Richard M. Myers[6], Lingyun Song[7], Gregory E. Crawford[7], [1]Human Genetics Program and Department of Biochemistry, Tulane Medical School, [2]Tulane Cancer Center, [3]Department of Mathematics, Tulane University, New Orleans, LA, USA, [4]University at Albany-SUNY, Albany, NY, USA, [5]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [6]HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA, [7]Institute for Genome Sciences & Policy, Duke University, Durham, NC, USA.

Facioscapulohumeral muscular dystrophy (FSHD) is a unique dominant disease linked to contraction of a tandem 3.3-kb repeat (D4Z4) at 4q35. A transcript from the *DUX4* homeobox gene in the most distal repeat unit is associated with the disease. However, the role of *DUX4* in pathogenesis is unclear. Exon-based microarrays were used to profile the expression of FSHD and control myoblasts and myotubes. Many genes with myogenesis- and FSHD-related changes in expression were identified by comparison of myoblasts, myotubes, and 19 different non-muscle cell types subject to identical expression profiling. By examining genome-wide DNA methylation and DNaseI-hypersensitive sites indicative of open chromatin, we identified epigenetic changes linked to some of this FSHD-associated dysregulation of RNA levels. Many genes encoding proteins involved in myogenesis, mitochondrial function, the oxidative stress response, and signal transduction were more than two fold dysregulated ($p<0.01$). FSHD-associated alterations in the gene expression profile for normal myogenesis were present at the myoblast stage as well as at the myotube stage. Although the disease-linked *DUX4* RNA isoform was detected by RT-PCR in about half of the examined FSHD myoblast and myotube preparations, it was present at only extremely low levels. Therefore, we propose that inappropriate expression of *DUX4* occurs transiently at an early stage in myogenesis, before myoblast formation, and sets up a cascade of dysregulation of other genes. Some of these newly identified FSHD-dysregulated genes are attractive targets for developing effective therapy for this disease and may be more amenable to therapeutic intervention than *DUX4* itself. (Supported in part by the FSHD Global Research Foundation [ME], the National Institutes of Health [NS04885 and AA3768G2 to M.E., HG003169 to G.E.C.], and the Fields Center for FSHD and Neuromuscular Research [R.T.]).

ORAL PRESENTATIONS

O1: NOVEL ANTI-HCV THERAPY: SINGLE SHRNA TARGETING BOTH STRANDS OF HCV

Leszek Lisowski[1], Menashe Elazar[2], Kirk Chu[1], Jeffrey S. Glenn[2] and Mark A. Kay[1], [1]Departments of Pediatrics and Genetics, [2]Departments of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA, United States, 94305.

Hepatitis C virus (HCV) is an enveloped RNA virus with a single-stranded RNA genome of (+) polarity, making it an attractive target for RNAi based therapies. However, its high rate of mutation-prone replication causes a great degree of genomic variability, which makes standard RNAi approaches ineffective. Like

other Flaviviridae, HCV replicates through a (-) strand intermediate, which is present in 20-100 times lower amounts than the (+) strand and may represent a more potent and/or a synergistic target with shRNAs intended for the (+) strand sequences. It is not known if the HCV (-) strand is bio-available for targeting, however, because both shRNA strands can potentially be loaded into the RISC complex, standard RNAi screens cannot distinguish between (+) and/or (-) strand targeting. We undertook a systematic approach to identify shRNA sequences capable of efficient knockdown of HCV positive and/or negative strands. To this end we designed 88 overlapping shRNAs staggered every 4bp targeting the most conserved regions of HCV. All shRNAs were cloned into rAAV constructs under the H1 promoter and tested against the (+) and (-) genome of HCV 2a using a luciferase reporter system, against replicating HCV-Luc and wtHCV. We then made asymmetrical shRNAs designed such that only one strand is able to perform RNAi cleavage. Using this approach we can for the first time experimentally demonstrate that the (-) strand is not accessible to standard RNAi-based therapy, indicating that the (+) and (-) strands of HCV genome are localized to different bio-compartments inside the infected cell.

O2: POTENTIAL TREATMENT OF 'LEAKY' DUCHENNE MUSCULAR DYSTROPHY MUTATIONS IN THE 5' REGION OF THE DYSTROPHIN GENE USING ANTISENSE OLIGOMERS

Carl F. Adkin, Abbie M. Adams, Kane Greer, Susan Fletcher and Stephen D. Wilton, Molecular Genetic Therapies Group, Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Verdun St, Nedlands, WA 6009, Australia.

Mutations in the dystrophin gene lead to the progressive muscle wasting disorder Duchenne Muscular Dystrophy (DMD). DMD mutations commonly disrupt the open reading frame or induce premature stop codons, leading to an absence of functional dystrophin protein. Antisense oligomer (AO) induced exon-skipping is a potential treatment, whereby the reading frame is restored by removal of exons during splicing, facilitating the translation of a truncated but semi-functional dystrophin protein. Here we present data showing that a subset of DMD mutations can be addressed by skipping dystrophin exon 8. These mutations are unusual in that they appear to be 'leaky' and can present as Becker Muscular Dystrophy (BMD) phenotypes. The genotype-phenotype discrepancy is suggested to be due to alternative splicing events or translation initiation from an alternative start codon in exon 8.

We have previously reported that targeting exon 8 induces skipping of both exon 8 and 9, and postulate that the splicing of these two exons is tightly linked. We demonstrate efficient skipping of exons 8 and 9 in cells from four DMD patients with amenable mutations, following treatment with both 2'O-methyl phosphorothioate AOs and phosphorodiamidate morpholino oligomers conjugated to cell penetrating peptides. We have also noted that targeting two AOs to exon 8 results in increased levels of exon 8 skipping only; however, the predominant transcript still lacks exon 8 and 9. BMD patients with a deletion of exons 3-9 have been reported with a very mild phenotype, exon skipping strategies that induce this dystrophin isoform could be therapeutically beneficial.

O3: REVERSAL OF DIABETES IN MICE FOLLOWING TRANSPLANTATION OF AN INSULIN-SECRETING LIVER CELL LINE: MELLIGEN CELLS

Ann M. Simpson[1], Chang Tao[1], Binhai Ren[1], M. Anne Swan[2], Bronwyn A. O'Brien[1], Paul Williams[3], [1]School of Medical & Molecular Biosciences, University of Technology

Sydney, [2]Anatomy & Histology[3] and Department of Endocrinology[4], University of Sydney, Sydney, Australia.

We are investigating gene therapy as a potential cure for Type I diabetes. An insulin-secreting cell line, Huh7ins which secreted insulin at sub-physiological glucose concentrations (2.5mmol/L), was further engineered to stably express human islet glucokinase: Melligen cells. The aim of the present study was to see if Melligen cells were responsive to glucose within the physiological range and if they would reverse diabetes in non-autoimmune nonobese diabetic severe combined immunodeficiency (NOD.*scid*) mice. Insulin storage and secretion were analysed by radioimmunoassay. Glucose phosphorylation was measured by following the conversion of [U-^{14}C] glucose to [U-^{14}C] glucose-6-phosphate (G-6-P). NOD.*scid* mice (n=6) were rendered diabetic by injection of streptozotocin and 1 x 10^7 Melligen cells were injected subcutaneously and diabetes reversal was monitored by blood glucose tests.

Melligen cells were responsive to glucose within the physiological range *in vitro:* 4.25 mmol/ L. The amount of insulin secreted to a glucose stimulus (0.48 ± 0.1 pmoles insulin/ 10^6 cells) was also double that of Huh7ins cells. Glucose phosphorylation assays indicated that most of the G-6-P activity was attributable to low K_m G-6-P-sensitive hexokinases in the Huh7ins cell line, whereas in Melligen cells, hexokinase activity represented 42% of the total glucose phosphorylating capacity, with the remainder from G-6-P-insensitive glucokinases. Blood glucose levels of diabetic mice that were transplanted with the Melligen cells became normal within 17-21 days. Blood glucose levels during the intra-peritoneal glucose tolerance test approached normal levels, peaking at 25 min (30 min for normal). Melligen cells are attractive candidates for surrogate beta cells.

O4: THE UTILIZATION OF GENE THERAPY OF HAEMATOPOIETIC STEM CELLS AS A STRATEGY TO TREAT AUTOIMMUNITY

Frank Alderuccio[1], Zeyad Nasa[1], Jieyu Chung[1], Amit Joglekar[1], James Chan[2] and Ban-Hock Toh[2], [1]Autoimmunity Laboratory, Department of Immunology, Central Clinical School, Monash University, Victoria, Australia, [2]Centre for Inflammatory Diseases, Department of Medicine, Southern Clinical School, Monash University, Victoria, Australia.

One of the major challenges for treating autoimmune diseases is the provision of a targeted therapy that can promote disease specific tolerance while maintaining normal immune function. From our understanding of the immune system suggest, exposure to antigen during development is an important and critical aspect of inducing tolerance. While expression by thymic epithelium is key to this, it has also been shown that bone marrow derived dendritic cells can also play a role in promoting antigen specific tolerance. With this in mind, we have pursued an experimental gene therapy strategy that involves *ex vivo* manipulation of bone marrow stem cells followed by transplantation to preconditioned recipients as a means of promoting antigen specific tolerance.

Experimental autoimmune encephalomyelitis (EAE) is a model of human multiple sclerosis and can be induced in mice by immunisation with myelin oligodendrocyte glycoprotein (MOG) or peptide (MOG_{35-55}). We have found that mice transplanted with bone marrow cells transduced with retrovirus encoding MOG generate bone marrow chimeras and fail to develop EAE following immunisation with MOG_{35-55}. We have extended this to an established disease model in which following remission induction and bone marrow transfer, mice do not relapse and remain disease free and tolerant. Notably, treatment with non-manipulated bone marrow does not

induce tolerance. We have shown that one mechanism of tolerance in this system involves deletion of antigen specific T cells within the thymus. We are extending our studies to fully elucidate the mechanisms of tolerance that may be engaged and also less-toxic regimes of preconditioning that may still promote chimerism and disease resistance. We believe that the ability to genetically manipulate and transfer modified bone marrow in a gene therapy approach offers a strategy to promote immune tolerance for the treatment of autoimmune disease.

O5: MIGRATION AND CELLULAR LOCALIZATION OF TRANSPLANTED HUMAN MESENCHYMAL STEM CELLS IN A MOUSE MODEL OF AUTOIMMUNE DEMYELINATION

Christopher Siatskas[1], Natalie Payne[1], Leon Moussa[1], Ashley Emerson Webber[1], John T. Price[2] and Claude C. Bernard[1], [1]Monash Immunology and Stem Cell Laboratories, Monash University and [2]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria.

Mesenchymal stem cells (MSCs) are promising vehicles for the treatment of neurodegenerative diseases such as multiple sclerosis (MS). This is in part due to their broad immunomodulatory properties and their ability to home to sites of tissue inflammation and injury. We have demonstrated that human MSCs can suppress the in vitro proliferation of stimulated lymphocytes and ameliorate the clinical signs of disease in mice with experimental autoimmune encephalomyelitis (EAE) – an animal model for MS. How MSCs home to the central nervous system– the site for tissue destruction in EAE, or other anatomical locations such as the peripheral lymphoid organs to impact disease progression has not been clearly defined. To understand this process in greater detail FACS analysis of molecules involved in cellular trafficking revealed that MSCs expressed a broad range of cell surface molecules involved in cellular adhesion including, integrins, tetraspanins and members of the immunoglobulin superfamily. Moreover semi-quantitative PCR confirmed the expression of a number of chemokine receptors. In vivo bioluminescent tracking of transplanted MSCs engineered to over express luciferase in immunocompetent Balb/c mice or immunocompromised NOD/SCID mice revealed that after intravenous infusion, most cells were trapped as emboli in the lung that persisted for approximately 3 to 4 weeks. In adjunct studies implementing two models of EAE, we demonstrate that transplanted gene modified MSCs only transiently localized primarily in the lung, or to a lesser extent in the spleen. Collectively, these results suggest that long-term engraftment of MSCs is not required for their enduring anti-inflammatory effects.

O6: ADVANCES IN CELLULAR THERAPEUTIC GENE TRANSFER TARGETING PROSTATE CANCER METASTASES

Jennifer M. Randall[1], Mark Tan[1], Michelle O'Han[2], Nham Tran[1], John E.J. Rasko[2,3,4], Stephen R. Larsen[2,3,4] and Rosetta Martiniello-Wilks[1], *[1]Translational Cancer Research Group, School of Medical and Molecular Biosciences, University of Technology Sydney; [2]Gene and Stem Cell Therapy Program, Centenary Institute and the University of Sydney; [3]Cell and Molecular Therapies, Royal Prince Alfred Hospital (RPAH); [4]Institute of Haematology, RPAH.*

Bone marrow derived mesenchymal stem cells (BMSC) are attractive cellular vehicles for cancer gene therapy (therapeutic BMSC) based on their ability to home to tumours and micrometastases *in vivo*. For *ex vivo* genetically-modified BMSC to be used in the clinic, it is essential to use methods that prevent therapeutic gene silencing and possible BMSC transformation. We employed

nucleofection (Lonza) for plasmid-based gene transfer that satisfies the Good Manufacturing Practice requirements for cell therapy development by the Therapeutic Goods Administration. BMSC were nucleofected with bicistronic plasmids containing a firefly luciferase and enhanced green fluorescent protein (fl:eGFP) fusion reporter gene alone or in combination with therapeutic gene yeast cytosine deaminase uracil phosphoribosyl transferase (CDUPRT) or murine interleukin 24 (mIL24). We tracked therapeutic BMSC distribution and efficacy in syngeneic RM1 prostate tumour bearing mice. The RM1 cells were labeled with renilla luciferase and red fluorescent protein (rl:RFP) fusion reporter gene and were implanted subcutaneously to form tumours or infused via the tail vein to establish lung pseudometastases. The fate of the BMSC was monitored by fl bioluminescence imaging (BLI) with substrate D-luciferase and RM1 PCa growth was monitored by rl BLI with substrate coelentrazine. We found that therapeutic BMSC migrated to RM1 conditioned media (CM) *in vitro* using transwell migration assays. Importantly, therapeutic BMSC significantly inhibited PCa tumour and metastases growth *in vivo*. Thus, therapeutic BMSC show promise as a cellular gene therapy for PCa metastases.

O7: TRANSIENT MOUSE MODELS FOR THE PRECLINICAL EVALUATION OF THERAPEUTIC DYSTROPHIN EXON SKIPPING STRATEGIES

Sue Fletcher, Abbie Adams, Carl Adkin, Kane Greer, Russell Johnsen and Steve Wilton, Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Perth.

Mutations that ablate dystrophin expression lead to Duchenne muscular dystrophy (DMD) an X-linked, relentlessly progressive muscle wasting disorder with a predictable course and limited treatment options. Corticosteroids are effective in stabilizing muscle strength in the short term but do not address the primary etiology of DMD, the absence of dystrophin. The majority of DMD cases are caused by frame-shifting deletions of one or dystrophin exons, while in-frame deletions generally cause the milder allelic disorder, Becker muscular dystrophy (BMD). Antisense oligomer (AO)-mediated splicing manipulation can exclude exons during transcript processing and by-pass DMD-causing mutations to generate shorter, partially functional BMD-like dystrophin isoforms, and is showing promise as a therapy for DMD. Dystrophin genes in selected BMD patients indicate templates for functional dystrophin isoforms, however, in-frame deletions in some regions of the dystrophin gene, particularly downstrean of exon 55 are rare, and the consequences of exon exclusion in this region are unknown. The *mdx* mouse is a widely used dystrophinopathy model and has a nonsense mutation in dystrophin exon 23. AO induced-excision of this exon from the mRNA removes the mutation without disrupting the reading frame, resulting in functional dystrophin expression and amelioration of the phenotype. We now report that systemic administration of AO combinations to wild-type mice can remove dystrophin exons to generate DMD- and BMD-like dystrophin isoforms for functional evaluation. Assessment of contractile properties of the muscle reveals that some in-frame exon combinations confer near normal function, while others result in muscle susceptible to contraction-induced damage.

O8: shRNA-MEDIATED INDUCTION OF SEVERE HYPERAMMONEMIA IN THE OTC-DEFICIENT SPF^{ash} MOUSE TO ASSESS THE CHALLENGES OF NEONATAL THERAPY WITH rAAV

Sharon C. Cunningham[1], Cindy Y. Kok[1], Afroditi Spinoulas[1], Samantha L. Ginn[1] and Ian E. Alexander[1,2], [1]Gene Therapy Research

Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, NSW, Australia. [2]Discipline of Paediatrics and Child Health, University of Sydney, NSW, Australia.

A major challenge when using rAAV gene therapy for the treatment of metabolic liver disease is maintaining persistent levels of therapeutic gene expression in a growing liver. We wish to address this challenge in the context of severe neonatal ornithine transcarbamylase (OTC) deficiency, a urea cycle disorder resulting in hyperammonemia. Using rAAV-mediated gene delivery we have successfully treated adult OTC deficient *spf*^{ash} mice (mild phenotype), normalizing orotic aciduria. Following neonatal vector delivery, however, only ~5% stable gene-modified cells remain at adulthood. While this was not sufficient to prevent orotic aciduria, it may be sufficient to prevent hyperammonemia. We have developed a model in which to test this, by using a shRNA to knock down endogenous OTC activity and create a severe phenotype. Neonatal *spf*^{ash} mice (1.5 days) were injected with rAAV2/8-LSP1.mOTC (2.5×10^{11} vg/mouse) and as adults (8-12 weeks) with rAAV2/8-shRNA-OTC (1×10^{12} vg/mouse). However, knockdown was inefficient as transduction was inhibited by an immune response to the initial injection. To overcome this, the shRNA was pseudo-serotyped with the AAV rh10 capsid. Knockdown was successful but despite achieving a level of 5-13% wildtype OTC activity, this was not sufficient for protection against hyperammonemia. Given that in the *spf*^{ash} mouse, OTC activity of ~5-7% is sufficient to protect against hyperammonemia under normal conditions, our experiments indicate that the proportion and distribution of gene-modified cells across the hepatic lobule is a critical factor in determining therapeutic success. Further experiments have been designed to establish the threshold of gene-modified cells required for protection against the severe OTC phenotype.

O9: NON-MYELOABLATIVE TRANSPLANTATION OF SELF-ANTIGEN EXPRESSING BONE MARROW PREVENTS AUTOIMMUNE DISEASE BY PERIPHERAL TOLERANCE MECHANISM

James Chan[1], Hamid Hosseini[1], Ding Yuan Oh[1], Siow T Chan[1], Xiang T Chen[1], Hideo Yagita[2], Frank Alderuccio[3] and Ban-Hock Toh[1], [1] Centre for Inflammatory Diseases, Department of Medicine, Southern Clinical School, Monash University, Clayton, Victoria, 3168, Australia, [2]Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan, [3]Department of Immunology, Central Clinical School, Monash University, Prahran, Victoria, 3181, Australia.

Transplantation of bone marrow (BM) engineered to express self-antigen under lethal myeloablative pre-conditioning protects 100% of mice from myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE). Here we asked whether transplantation of MOG expressing bone marrow (pMOG-BMT) under non-myeloablative pre-conditioning provides the same protection. Using stepwise reduction of irradiation doses, 275 cGy irradiation with pMOG-BMT protected 100% of mice from developing MOG-induced EAE even upon subsequent re-challenge with MOG. Irradiation doses lower than 275 cGy produced partial protection in a dose-dependent manner. At 50 cGy, pMOG-BMT provided significant disease protection compared to controls. Splenocytes from 275 cGy recipients proliferated in response to MOG stimulation in vitro indicating that MOG-reactive cells were present in the peripheral but failed to induce disease. However, the MOG-stimulated cells produced little or no IL-17, IFNγ, GM-CSF and TNFα compared to EAE control. Adoptive transfer of total CD4 T cells from mice resistant to EAE even after 2 rounds of MOG

immunization into Rag2$^{-/-}$ mice resulted in MOG-induced EAE in ~74% of mice. These data suggest that non-myeloablative transplantation of BM engineered to express self-antigen induces resistance to EAE development through peripheral tolerance.

O10: DEVELOPMENT AND APPLICATION OF SITE-SPECIFIC INTEGRATION OF FUNCTIONAL GENOMIC LOCI

Jim Vadolas, Bradley McColl, Sara Howden and Kasey Chan, Cell and Gene Therapy Research Group, Murdoch Childrens Research Institute, The University of Melbourne, Royal Children's Hospital, Parkville 3052, Melbourne, Australia.

The availability of gene transfer systems that permit long-term expression of a therapeutic transgene and avoid the risks associated with random integration is crucial to the further development of gene therapy protocols. We focused our efforts on improving gene therapy protocols, which combine two lines of investigation: 1) Targeted integration of therapeutic genes at safe sites within the human genome, thereby avoid insertional mutagenesis, and 2) the use of intact functional genomic loci to achieve physiological regulated transgene expression. We developed a novel strategy for the targeted integration of bacterial artificial chromosomes (BAC) vectors containing functional genomic loci. BACs containing the P5 integration efficiency element (P5IEE) derived from adeno-associated virus (AAV) were targeted to integrate specifically into chromosome 19, designated AAVS1. This was achieved using AAV Rep 68/78 mRNA, which codes for the AAV integrase. Co-transfection of a 21 kb BAC containing P5IEE with Rep mRNA facilitated the targeted integration into the AAVS1 site in up to 32% of all clones. Using this approach, the site-specific integration of a 200 kb BAC, containing P5IEE and carrying the entire human β-globin locus was used to transfect K562 cells. We identified several K562 clones, containing the β-globin locus integrated at the AASV1 site, at 20% efficiency. Our study suggests that Rep mRNA transfection can be used to facilitate the site-specific integration of genomic loci. We propose that this gene therapy strategy may be used in conjunction with patient-derived stem cells to facilitate persistent and stable transgene expression. Alternatively, this technology opens the door to the systematic generation of reporter stem cell lines allowing fine-tuning of differentiation protocols in order to apply stem cell technology to a variety of pathological conditions.

O11: SUCCESSFUL IN VIVO GENE THERAPY FOR A WHITE MATTER DISEASE AFTER ADENO-ASSOCIATED VIRUS VECTOR DELIVERY TO OLIGODENDROCYTES

Nadine Mersmann [1], Eva-Maria Krämer-Albers[2], Orla Teahan [3], Matthias Klugmann [1,3], [1]Department of Physiological Chemistry, University Medical Centre, Johannes Gutenberg University, Mainz, Germany; [2]Department of Biology, Molecular Cell Biology, Johannes Gutenberg University Mainz, Mainz, Germany; [3]Translational Neuroscience Facility, Department of Physiology, School of Medical Sciences, University of New South Wales, Sydney, Australia.

Canavan disease (CD) is an early onset neurological disorder caused by a null mutation in the gene encoding the enzyme aspartoacylase (ASPA). ASPA is normally enriched in oligodendrocytes, the myelin forming cells in the central nervous system (CNS), and ASPA-deficiency results in oligodendrocyte dysfunction, brain vacuolization, and mental and physical retardation. CD has been the prototype of a gene therapy for neurogenetic disorders because of its monogenic nature, a pathology confined to the CNS and the possibility of a

gene replacement approach. Adeno-associated virus (AAV)-mediated delivery of the ASPA gene to patients or animal models had failed in the past amid the inherent neurotropism of AAV vectors. To overcome this limitation, we have employed promoter targeting to develop recombinant AAV for efficient transduction of oligodendrocytes. To test the therapeutic potential of this novel vector system, we delivered AAV-ASPA or empty vector (2×10^{12} vg/ml) to the striatum, thalamus and cerebellum of ASPA-deficient mice at the age of 10 days. Untreated wild-type littermates served as control. Our results showed that ASPA protein levels were restored in oligodendrocytes of AAV-ASPA treated mutants. Brain vacuolization was absent even in regions distant from the injection sites and the body weight was normalized. Motor performance and complex social behaviour were fully restored in mutants after AAV-ASPA treatment while empty vector treatment had no effect. Overall, we provide evidence of the first successful gene therapy for CD. Our findings may well be extended to other white matter diseases or whenever efficient gene transfer to oligodendrocytes is required.

O12: SEQUENCE SPECIFIC INDUCTION OF HIV-1 TRANSCRIPTIONAL GENE SILENCING TARGETING NF-κB SEQUENCES BY SIRNA WITHOUT ANY OFF-TARGET EFFECTS OF OTHER NF-κB DRIVEN GENES

K Suzuki[1,2], S Swaminathan[1,2], C Hood [1,2], DA Cooper [1,2], T Watanebe [2], AD Kelleher [1,2], [1]St Vincent's Centre for Applied Medical Research, Australia, [2]National Centre in HIV Epidemiology and Clinical Research, UNSW, Australia.

We have previously reported the induction of prolonged HIV-1 transcriptional gene silencing (TGS) by shRNAs targeting the highly conserved tandem NF-κB binding sequences within the HIV-1 promoter region. Recent articles have suggested that siRNAs based approach often result in induction of off-target effects. We investigated sequence specific induction of TGS using retroviral delivery system. We established MOLT-4 cell lines stably expressing an shRNA targeting NF-κB (termed MOLT-4 shPromA), four variants expressing shRNA with 2-3 mismatches, and a scrambled control. We confirmed presence of processed double-stranded siRNAs from shRNA transcripts in all MOLT-4 cells by RT-PCR and also confirmed that these were expressed at similar levels in each of the MOLT-4 cell lines. MOLT-4 shPromA did not alter expression of any of the other 86 NF-κB driven genes including IFN(α1, β1, γ) and 5 house keeping genes (β2M, HPRT1, RPRL13A, GAPDH, βActin). Sequence specific HIV-1 suppression was achieved only in MOLT-4 shPromA cells, in which perfect matched siRNA to HIV-1 promoter region is expressed. The 2-3 mismatched shPromA failed to suppress HIV-1 replication. Our data demonstrate that shPromA induces sequence specific transcriptional silencing in HIV-1 gene, not altering other NF-κB driven genes. Alternation of three IFN-α response genes and PKR activation were not observed in MOLT-4 shPromA cells. MOLT-4 shPromA achieved sustained and highly specific HIV transcriptional gene silencing. Our study demonstrates that transcriptional gene silencing may represent a new therapeutic approach with minimal off target effects from NF-κB driven genes.

O13: CO-REGULATION OF THE PROGRAMMED CELL DEATH PROTEIN 4 (Pdcd4) BY miR-21 and miR-499

Nham Tran [1, 2, 3], Xiaoying Zhang [1, 2], Barbara Rose [1, 2], and Rosetta Martiniello-Wilks[3], [1]The Sydney Head and Neck Cancer Institute, Sydney Cancer Centre, Royal Prince Alfred Hospital and University of Sydney, Sydney,

NSW, Australia, [2]Department of Infectious Diseases and Immunology, University of Sydney, Sydney, NSW, Australia, [3]Translational Cancer Research Group. School of Medical & Molecular Biosciences, University of Technology Sydney, NSW, Australia.

MicroRNAs are small non coding RNA molecules which control gene expression and dysregulation of the miRNA milieu is associated with head and neck cancer. Several studies have reported on the expression profiles in head and neck cancer but one of the main challenges is to understand the role of these dysregulated miRNAs in head and neck tumorigenesis. Here we report on a non-coding miRNA profiling study using oropharyngeal squamous cell carcinoma, a common subtype of head and neck cancer. Eleven miRNAs were dysregulated by more than 2-fold including miR-499 and miR-21. We demonstrated that both miRNAs temporally regulate PDCD4 at the post-transcriptional level. The initial suppression of PDCD4 was mediated by miR-21 whilst sustained suppression was mediated by miR-499. Moreover the single miR-21 site was able to elicit the same magnitude of suppression as the three miR-499 sites. Immunohistochemistry showed that PDCD4 expression was significantly reduced in cancers relative to normal cells. Our findings have added a new level of complexity to miRNA mediated gene regulation and suggests that the dual downregulation of the tumour suppressor PDCD4 by miR-499 and miR-21 acting in concert may be an important molecular event in the development of head and neck cancer.

O14: ANTAGOMIR-MEDIATED INHIBITION OF INFLAMMATION

Michael P. Gantier[1], H. James Stunden[1], Claire E. McCoy[1], Mark A. Behlke and Bryan R.G. Williams[1], [1]Centre for Cancer Research, Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia; [2]Integrated DNA Technologies, Coralville, Iowa, USA.

MicroRNAs (miRNAs) are a new class of small RNAs regulating gene expression, critical for normal cell function. Relying on the inducible genetic ablation of *Dicer1* and subsequent miRNA depletion, we demonstrate an important role for miRNAs in the control of pro-inflammatory cytokine production in cells. Antisense-mediated inhibition of miRNA function by antagomiR confirmed the involvement of select miRNAs in the positive control of NFκB signalling. Through the use of *in silico* predictions and reporter assays we show that these miRNAs negatively control expression of A20 (TNFAIP3), resulting in increased NFκB signalling. AntagomiR-mediated inhibition of these miRNAs results in inhibition of pro-inflammatory cytokine production in cell lines. These results suggest that antagomiRs could present novel therapeutic avenues to control inflammatory disorders.

O15: THE POTENTIAL AND LIMITATIONS OF MANIPULATING TGF-β SIGNALLING TO TREAT MUSCLE WASTING

Paul Gregorevic, Laboratory for Muscle Research & Therapeutics Development. BakerIDI Heart and Diabetes Institute, Melbourne 3004, Victoria, Australia.

Loss of skeletal muscle mass and strength is a common and significant contributor to impaired health and premature mortality. In skeletal muscle, the TGF-β signalling is a prominent regulator of development and post-natal adaptation (especially control of cell size). Thus, strategic manipulation of cellular events that are governed by TGF-β signalling may provide a means to prevent or treat muscle-related disease. Our lab uses recombinant adeno-associated viral vectors to alter expression of TGF-β family ligands,

ligand binding proteins, components of the intra-cellular signalling pathway, and target genes, to evaluate novel strategies for preventing skeletal muscle wasting. In mice, we have observed that local expression of follistatin isoforms (as myostatin and activin A inhibitors) can promote a 100% increase in muscle mass and >50% increase in maximum force producing capacity within 4 weeks of treatment. Moreover, animals treated via systemic rAAV vector administration exhibit a similar hypertrophic response body wide, which is sustained without further intervention for 24+ months in a manner that can compensate for the loss of muscle mass otherwise observed in old mice. We have determined that muscle hypertrophy caused by this intervention is associated with suppression and stimulation of Smad-dependent signaling, which controls an extensive program of gene expression in skeletal muscle. By developing vectors that target specific aspects of Smad signaling, we can stimulate skeletal muscle hypertrophy independent of follistatin expression. In subsequent studies, we have also determined that the key signaling and transcriptional events that occur during follistatin-mediated muscle hypertrophy do not depend on the IGF-I/Akt/mTOR axis, but can be augmented by vectors that co-stimulate this pathway. As a prospective approach for combating frailty resulting from muscle wasting and dysfunction in disease, we have examined the effects Fst-288 expression in animal models of muscle-related disease and observed differing responsiveness to acute follistatin expression. We propose these distinct outcomes reflect the direct and indirect involvement of the TGF-β pathway in muscle wasting conditions of differing etiology. This communication will provide an overview of our understanding of the mechanisms associated with skeletal muscle adaptation mediated by TGF-β signalling, and consider the potential for developing TGF-β pathway-based therapeutic interventions for muscle-related disease.

O16: TRANSFER OF AAV-SPECIFIC MATERNAL ANTIBODIES IN BREAST MILK IMPEDES VECTOR DELIVERY TO JUVENILE MICE

Cindy Y. Kok[1], Sharon C. Cunningham[1], Ian E. Alexander[1,2], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, NSW, Australia, [2]Discipline of Paediatrics and Child Health, University of Sydney, NSW, Australia.

The citrullinaemic mouse is a knock-out model for argininosuccinate synthetase (ASS) deficiency and has a neonatal lethal phenotype resulting from severe hyperammonaemia. Using this model, we have shown that pre-natal gene transfer of an adeno-associated viral vector (rAAV.ASS) with re-administration at 0, 2 or 4 weeks after birth was necessary to extend survival to 5 weeks. Each additional dose resulted in only a small increase of ASS activity, with a maximum of 5.4% wildtype activity achieved. We hypothesised that vector-specific antibodies from dams exposed to rAAV.ASS may have been transferred through breast milk to the pups and thus reduced transduction efficiency of subsequent vector re-administration. To test this hypothesis, pregnant dams were injected at E16 with 10^{12}vg of rAAV.GFP or PBS. The resulting pups were cross fostered so that naive pups were exposed to milk from the injected mothers. These pups were then injected with 2.5 x 10^{11}vg of rAAV.GFP at two weeks of age and harvested after 7 days to analyse antibody titre in serum and expression of GFP in the liver. Control pups which were fostered to immunised dams had detectable levels of antibodies to the vector. These titres were sufficient to block gene transfer following injection of rAAV.GFP. This observation confirmed that maternal antibodies transferred to pups through the

breast milk were capable of impeding subsequent gene transfer. Future treatment which involves vector redelivery following *in utero* injection will require fostering of neonatal treated pups to naive mothers.

O17: ENHANCED NUCLEAR TRANSPORT IN TRANSFORMED CELLS; PROSPECTS FOR ANTI-CANCER GENE THERAPY?

Henna V. Kuusisto, Kylie M. Wagstaff, Gualtiero Alvisi and David A. Jans' Nuclear Signalling Laboratory, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.

Despite an abundance of highly cytotoxic drugs/genes, our ability to treat cancer remains limited, meaning that there is an urgent need for approaches to maximize tumour killing activity, whilst sparing the destruction of healthy cells during therapy. Targeted delivery of drugs/genes into the nucleus through exploiting the cellular superfamily of Importin (Imp) transporters appears as a possibility in this context, based on the fact that specific Imps are overexpressed in a variety of tumour cells/tissues. The efficiency of nucleocytoplasmic transport in tumour compared to normal cells, however, has not previously been assessed. In this study we used quantitative live cell imaging to show that the nuclear accumulation of nuclear localization signal (NLS)-containing proteins is globally enhanced in several Simian Virus 40 (SV40)-transformed cells, compared to their non-transformed isogenic counterparts. This does not appear to result from the inhibition of nuclear export or altered exclusion properties of the nuclear pore through which nuclear transport is mediated; rather, photobleaching experiments indicate that the basis of this is a significantly faster rate of nuclear import in the transformed cells. Comparable results were obtained in the non-virally transformed isogenic MCF10A/AT/CA1 breast tumour progression model, with quantitative analysis suggesting that the degree of enhancement of nuclear import correlates with that of tumour progression. Importantly, our Western and densitometric analysis show that enhanced nuclear import efficiency is associated with overexpression of specific Imps in both SV40- and non-virally-transformed cells. The findings have exciting application in improving tumour cell-selective delivery of nuclear-acting drugs/genes in non-viral anti-cancer therapy.

O18: INVESTIGATING TRANSGENE EXPRESSION ACROSS THE HEPATIC LOBULE AFTER RAAV MEDIATED GENE DELIVERY

Allison Dane[1], Sylvia Wowro[1], Sharon Cunningham[1], and Ian Alexander[1,2], [1]Gene Therapy Research Unit, The Children's Hospital at Westmead and Children's Medical Research Institute, Westmead, NSW 2145, Australia. [2]Discipline of Paediatrics and Child Health, University of Sydney, NSW 2000, Australia.

Genetic disorders of the urea cycle, whilst individually rare, are collectively common and difficult to treat. Liver transplantation is often required, but carries its own set of risks and limitations. Gene therapy presents an alternative therapeutic approach. Recombinant adeno-associated virus (rAAV) vectors have shown initial promise for liver directed gene therapy, due to their efficacy in small and large animal models. However, further understanding of host-vector interactions, especially those controlling initial target cell specificity and transduction is required.

We have observed that mice dosed with rAAV2/8.LSP1.eGFP exhibit a differential pattern of eGFP expression across the hepatic lobule, with higher levels of expression in the perivenous zone. This observation is important considering that many hepatic genes

are differentially expressed across the hepatic lobule. The urea cycle enzymes are expressed predominantly in the periportal zone with a decreasing gradient of expression toward the perivenous zone. The aim of this study was to develop vectors that would preferentially target periportal hepatocytes.

We compared the expression patterns of AAV vectors pseudoserotyped in alternative capsids (type 2, 7, 8 9 and rh10) as well as vectors employing different liver-specific promoter/enhancer elements. Post-transcriptional control of transgene expression pattern by microRNAs was also investigated. While the total level of eGFP expression varied across the panel of vectors bearing different transcriptional control units, the perivenous pattern of expression remained. Capsid choice, however, was observed to dramatically influence the pattern of expression. These results highlight the importance of considering both capsid type and promoter/enhancer choice when designing and optimising vectors to preferentially target specific zones of the hepatic lobule.

O19: MICROSTRUCTURED HYBRID CONDUCTING POLYMER PLATFORMS FOR SKELETAL MUSCLE

RMI Kapsa[1,2], Razal JM[1], Quigley AF[1,2], Kita M[1,2], Mysore T[1,2], Moulton S[1], Penington AJ[3], Clark GM[4] and Wallace GG[1], [1]ARC Centre of Excellence for Electromaterials Science, (IPRI), Wollongong, NSW 2522, Australia, [2]Centre for Clinical Neuroscience and Neurology Research, St. Vincent's Hospital, Fitzroy, VIC 3065, Australia, [3] Bernard O'Brien Institute for Microsurgery, Fitzroy, Vic., 3065, Australia, [4]Hearing and Neuroscience Unit, School of Physiological Science, Latrobe University, Bundoora, VIC, Australia.

The 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. We have been investigating the use of microstructured conducting polymer surfaces for the orientation and electrical stimulation of cells and tissues and have developed a hybrid biodegradable and conducting polymer platform suitable for *ex vivo* muscle growth and differentiation.

In this study, wet spun biodegradable polymer fibres were deposited onto gold mylar at controlled intervals. Interfibre distances between 50-600 microns were created on the platforms. To create a conductive microstructured hybrid platform, a layer of polypyrrole was deposited in between the aligned wet-spun biodegradable polymer fibres, on a sheet of gold mylar[1]. Myoblasts extracted from 8 week old mice were then seeded onto polymer platforms and induced to differentiate into myotubes. Analysis of myofibre differentiation and orientation was then performed.

Platforms were developed to facilitate the alignment and differentiation of skeletal muscle stem cells (myoblasts) to reflect the orientation of mature skeletal muscle. Microstructured platforms were developed by spatially orientating 30 micron diameter PLGA polymer fibres on a conducting polymer platform at varying intervals. Human and murine myoblasts could be grown on these platforms without the use of cell adhesion molecules. PCNA staining revealed the presence of proliferating myoblasts on both the biodegradable polymer fibres, as well as the conducting polymer surface. Fibre spacing was found to have a significant effect on the extent of myotube orientation, with increased orientation seen with decreasing inter-fibre distance. Myotube alignment was most pronounced when inter-fibre distances were less than 160 microns and diminished significantly with increasing inter-fibre distances. A hybrid platform with fibre spacing less than 20 microns confined the

myotubes entirely to the biodegradable fibres and resulted in the alignment of great majority of the fibres.

These studies demonstrate that novel hybrid platforms can be used to influence skeletal muscle differentiation *ex vivo*. In addition these platforms can influence muscle fibre orientation in a manner reflecting the *in vivo* architecture of the parent tissue. Such platforms have application for controlling the regeneration of skeletal muscle *in vivo* and for the integration of bionic devices designed to facilitate muscle regeneration and function.

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O20: MICRORNAS AS TOOLS TO TARGET BONE MARROW MEDIATED TUMOUR ANGIOGENESIS

P.N. Plummer[1], R. Taft[2], J. Mattick[2], N. McMillan[3], A. Swarbrick[4], R. Brink[4], V. Mittal[5], A.S. Mellick[1], [1] Host Response to Cancer Lab, School of Medical Science, Griffith University, Gold Coast, QLD,[2]Institute of Molecular Biosciences, University of Queensland, Brisbane, QLD,[3]Diamantina Institute, University of Queensland, Brisbane, QLD,[4]Garvan Institute of Medical Research, Sydney, NSW,[5]Medical College, Weill Cornell University, New York, NY, United States.

Targeting the process of tumour vascularization is a key aspect of modern anticancer therapy. However, the cellular and molecular mechanisms by which tumours enroll vasculature remains relatively undefined. Furthermore, the role of host inflammatory cells, and bone marrow derived (BMD) cells in this process, despite extensive research, remains controversial. BMD endothelial progenitor cells (EPCs) have been identified by our lab and others as being critical for the angiogenic switch[1-3]. However, because different markers are used to track these cells in the bone marrow, blood and tumour-stroma, controversy exists as to whether the same cell is truly being followed *in vivo*. Using a lentiviral and transgenic mouse model we have the Inhibitor of DNA Binding 1 (Id1) can be used to mark and track EPCs from the BM to their incorporation as part of the tumour vasculature[1]. This work also demonstrated that Id1 can be used to target EPCs and suppress tumour angiogenesis. Small RNA Illumina analysis has identified small non-coding RNAs in particular microRNAs (miRNA) which distinguish tumour vasculature from lung vasculature; as well as specific changes in BM lineages following tumour challenge. Fluorescent miRNA In-situ Hybridization has also been used to demonstrate localization of several of these microRNAs to tumour vasculature and EPCs.

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O21: GENERATION AND ANALYSIS OF A HIGHLY COMPLEX Γ-RETROVIRAL VECTOR INTEGRATION SITE DATASET FROM HUMAN CD34+ CELLS USING ILLUMINA NEXT-GENERATION SEQUENCING

Claus V. Hallwirth[1], Jessica Hyman[2], Gagan Garg[3], Xiaoan Ruan[4], Lavanya Veeravalli[4], Belinda Kramer[2], Atif Shahab[4], Chin Thing Ong[4], Christopher Liddle[5], Shoba Ranganathan[3], David W. Russell[6], Chia-Lin Wei[4,7], and Ian E. Alexander[1], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, Australia; [2]Children's Cancer Research Unit, The Children's Hospital at Westmead, Westmead, Australia; [3]Department of Chemistry and Biomolecular Sciences, Macquarie University, Macquarie

Park, Australia; [4]Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore; [5]Department of Clinical Pharmacology, Sydney Medical School, University of Sydney, Sydney, Australia; [6]Department of Hematology, School of Medicine, University of Washington, Seattle, USA; [7]Department of Energy Joint Genome Institute, Walnut Creek, California, USA.

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. The foremost constraint underlying these analyses is the limited complexity of all but the fewest of integration site datasets generated thus far. In this study, DNA from human $CD34^+$ progenitor cells transduced under conditions relevant to contemporary clinical trials was used to generate a library of vector/genomic DNA junction fragments, using an extensively modified ligation-mediated PCR approach linked to Illumina next-generation sequencing (NGS). Numerous custom bioinformatics approaches were developed to facilitate correct base calling, and the retrieval and mapping of integration sites from the resulting data, leading to the generation of the most complex vector integration site dataset generated from a single NGS run, and the first reported use of Illumina NGS technology in this context. The known genome-wide integration preferences of γ-retroviral vectors were confirmed, but in addition, the complexity of the dataset provided the capacity to examine additional parameters, such as integration frequencies in and near loci involved in lymphoid oncogenesis. The methodology developed therefore permits analyses of vector integration not only at a genome-wide scale, but at the single-gene level.

O22: PERSONALISED GENETIC THERAPIES FOR NEUROMUSCULAR DISEASES

Steve Wilton and Sue Fletcher, Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Australia.

An estimated 75% of human genes undergo some form of alternative splicing to increase genetic plasticity and we can exploit this flexibility in pre-mRNA processing by inducing specific mRNA spliceoforms with antisense oligomers. These splice-switching compounds interfere with spliceosome assembly by masking motifs (splice sites, enhancers or silencers) involved in exon recognition and inclusion in the mature mRNA. Targeted exon skipping, induced during the processing of a defective dystrophin pre-mRNA, can bypass the protein-truncating disease-causing mutation in the mature mRNA and allow translation of a functional protein. Restoration of dystrophin expression after targeted exon skipping has progressed from a concept to the recent completion of two Phase I/II clinical trials in little over a decade.

Different oligomer chemistries are being evaluated, and independent trials have restored some dystrophin expression in participating DMD individuals. Although encouraging results have been reported, there are many challenges ahead including:

- establishing appropriate dosing regimens.

- bringing many different oligomers to the clinic and developing appropriate safety/toxicology packages.

- affordability and sustainability.

If exon skipping is shown to benefit DMD patients, there will be great impetus to apply oligomer-induced alternative splicing to other genetic disorders, including spinal muscular atrophy, where an exon needs to be retained in the mature transcript (achieved by masking intronic silencer elements). Disruptive splicing or induction of frame-shifts in a targeted mRNA could be used to suppress inappropriate expression of genes implicated in autosomal dominant disorders. Future splice switching applications may only be limited by our imagination.

POSTER PRESENTATIONS

P1: CAN 'EXON-SKIPPING' AMELIORATE THE PHENOTYPE OF MUTATIONS IN DYSTROPHIN EXON 74?

Carl F. Adkin, Kane Greer, Abbie M. Adams, Susan Fletcher and Stephen D. Wilton, Molecular Genetic Therapies Group, Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Verdun St, Nedlands, WA 6009 Australia.

Duchenne Muscular Dystrophy (DMD) is caused by mutations in the dystrophin gene that ablate dystrophin expression. Approximately 80% of DMD mutations can potentially be treated by antisense oligomer (AO) induced exon skipping to facilitate translation of dystrophin. However, in some cases, the nature of the mutation is such that exon skipping would not be applicable because it impacts upon crucial functional domains. Analysis of the mutations in Becker Muscular Dystrophy (BMD) patients has indicated templates for internally deleted, but potentially functional dystrophin isoforms. Our goal is to develop AOs to make exon skipping available to all DMD patients with potentially amenable mutations, including those with rare mutations.

Mutations within dystrophin exon 74 are relatively rare; however, there are currently 2 families in Western Australia with such mutations. These mutations are intriguing as small rearrangements, that would be predicted to induce a severe DMD phenotype, present with range of severity, from severe DMD to mild BMD in terms of muscle phenotype and variable neurological involvement in these patients. In some cases, identical mutations lead to very different severities, even within a family. This variability has been attributed to alternative splicing of exons around exon 74 or to inefficient nonsense mediated decay of the truncated transcript. Here, we report AOs that induce very efficient removal of exon 74 in human and mouse myogenic cultures. If the removal of exon 74 can be shown to generate a functional dystrophin isoform, this presents a potential treatment strategy for a subset of DMD patients.

P2: MURINE SOMATIC CELL REPROGRAMMING TO PLURIPOTENCY AND DIFFERENTIATION INTO HEPATOCYTES

Gustavo de Alencastro[1, 2], Grant J. Logan [1], Christine Smyth[1], Joshua B. Studdert[2], Kirsten A. Steiner[2], Patrick P.L Tam [2] and Ian E. Alexander [1], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, University of Sydney, Sydney, Australia; [2]Embryology Unit, Children's Medical Research Institute, University of Sydney, Sydney, Australia.

For metabolic liver disease, such as OTC deficiency which is the most common disorder of the urea cycle, the only treatment available is liver transplantation. However, the number of patients requiring transplantation exceeds the availability of donor organs. The associated surgical risks, requirement for life-long immune suppression and high costs involved make the search for alternative treatments an urgent priority. Autologous

hepatocytes derived from induced pluripotent stem cells (iPSCs) potentially bypass the need for immune suppression and offer a promising option, but protocols for the differentiation of functionally competent hepatocytes have yet to be developed. The focus of this project is to define the culture parameters for generating mature hepatocytes from murine iPSCs (miPSCs). This will be facilitated by the use of spectrally distinct fluorescent reporters under the control of lineage-specific enhancer/promoter of *Sox17* (definitive endoderm marker), *α-fetoprotein* and *Albumin* (immature and mature hepatocyte markers, respectively). For directed hepatocyte differentiation, the ability to visualize individual cells as they differentiate will allow tracking and selection of cells that display optimal hepatic differentiation. To date, we have reprogrammed miPSCs from embryonic fibroblasts by expressing the four Yamanaka factors using the Lenti-STEMCCA vector (Sommer *et al*., 2008). Preliminary results have shown that at day 4 of differentiation, pluripotent genes (*Nanog* and *Oct4*) were down-regulated and endodermal markers (*Sox17* and *FoxA2*) were up regulated. At the last day of differentiation (day 23), *albumin* and *alpha-fetoprotein* were upregulated with concomitant down regulation of the endodermal markers that is characteristic of hepatocyte differentiation.

P3: OFF-TARGET EFFECTS AS A CONSEQUENCE OF SPLICE SWITCHING ANTISENSE OLIGOMER ADMINISTRATION

Soma Amin, Lucy Barett, Sue Fletcher and Steve Wilton, Australian Neuromuscular Research Institute, University of Western Australia, Perth, WA, Australia.

Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by mutations in the dystrophin gene. DMD is a common and serious form of childhood muscle wasting that affects 1 in 3500 males. Antisense oligomers can be designed to interfere with normal dystrophin pre-mRNA processing so that a targeted exon is masked from the splicing machinery and excluded from the mature mRNA. In this way exons carrying a nonsense mutation or exons flanking frame shifting deletions are excluded from the gene transcript so a shorter but functional dystrophin is expressed. Studies on a mouse model of muscular dystrophy, carrying a nonsense mutation in dystrophin exon 23 have shown that targeted exon removal can restore dystrophin expression. Here, we examine muscle RNA from *mdx* and wild type mice, and *mdx* mice treated with different oligomers, to confirm changes in gene expression previously identified by proteomic profiling and microarray studies. Human homologues of genes showing altered expression in response to the presence or absence of dystrophin in the mice will then be studied in normal and DMD human muscle samples. Confirmation of particular differences in gene expression, between normal and dystrophic muscle, will help to assess 'normalization' of gene expression in response to treatment with splice switching oligomers that restore dystrophin expression, and may identify off target effects.

P4: THE NOVEL OUTCOME OF SMN OVER-EXPRESSION ON BCL-X SPLICING

Ryan S. Anderton [1], Loren Price [1], Wai Mitrpant [1], Bruno P. Meloni [1, 2], Bradley J. Turner [3], Frank L. Mastaglia [1], Steve D. Wilton [1], Sherif Boulos [1], [1]Centre for Neuromuscular and Neurological Disorders, University of Western Australia; Australian Neuromuscular Research Institute, Western Australia, [2]Department of Neurosurgery, Sir Charles Gairdner Hospital, Western Australia, [3]Neurodegeneration Division, Florey Neurosciences Institute, Victoria.

Purpose: Spinal muscular atrophy (SMA), a neurodegenerative disorder primarily affecting

motor neurons, is the most common genetic cause of infant death. This incurable disease is caused by the absence of a functional *SMN1* gene which leads to a critical reduction in full length SMN protein. The SMN protein has been linked to numerous cellular functions, including snRNP assembly and suppression of apoptosis. However, exactly how SMN protein imparts its cell survival function remains unknown. This study aimed to characterise the anti-apoptotic properties of SMN and compare them to those of the Bcl-xL protein.

Methods: Recombinant adenoviral vector mediated over-expression of SMN and Bcl-xL in SH-SY5Y cells and patient fibroblasts was investigated. In addition, the relationship between SMN and Bcl-xL was assessed using real-time RT-PCR and western blot analysis *in* vitro, and in a transgenic mouse model of SMN (Prp-SMN).

Results: SMN over-expression significantly increased Bcl-xL levels in SH-SY5Y cells and in PrP-SMN mice. Interestingly, PrP-SMN mice showed altered Bcl-x splicing, which favoured the production of the longer neuroprotective isoform of the Bcl-x protein. Bcl-xL over-expression also increased SMN levels 2.5 fold, suggesting combinational therapies that up-regulate both SMN and Bcl-xL would be beneficial. In addition, real-time analysis has demonstrated exogenous addition of SMN can increase endogenous SMN levels, indicating that even small increases in FL-SMN expression may lead to the induction of therapeutic SMN levels.

P5: MANIPULATION OF DYSTROPHIN EXPRESSION USING ANTISENSE OLIGONUCLEOTIDES TARGETED TO THE 3' UNTRANSLATED REGION

Lucy Barrett, Loren Price, Sue Fletcher and Steve Wilton, Centre for Neuromuscular and Neurological Disorders, University of Western Australia.

Regulation of gene expression is a complex and tightly controlled process in which the 5' and 3' untranslated gene regions play a major role. The 5' untranslated region is a vital to translational control, while the 3' untranslated region influences transport and subcellular localisation of the mRNA, and is the major determinant of transcript stability. Manipulation of gene regulation at the mRNA level could provide therapeutic benefit to patients with diseases in which mRNA levels are reduced or the message is unstable, leading to reduced levels of protein. Duchenne muscular dystrophy (DMD) is caused by protein-truncating mutations in the dystrophin gene that lead to nonsense mediated decay of the gene transcript and subsequent absence of functional protein. Becker muscular dystrophy (BMD) is a milder allelic condition that also results from mutations in the dystrophin gene, however these mutations do not disrupt the reading frame. The expression levels and function of the protein (and thus the disease severity) depend on the nature and location of the mutation. A promising treatment for DMD currently in clinical trials involves the correction of the reading frame using antisense oligonucleotides (AOs) to redirect splicing and remove targeted exons from the mature dystrophin mRNA, thereby bypassing truncating mutations. This therapy should allow the production of a semi-functional protein, as expressed in BMD patients, from a DMD gene. As many BMD patients show symptoms of muscle weakness and degeneration, it is likely that they would benefit from increased dystrophin levels. In addition, some other therapies under evaluation (read-through of nonsense mutations) should also benefit from increased dystrophin mRNA levels. We show that AOs targeted to the 3' untranslated region of the dystrophin gene can increase mRNA levels 2-fold in normal human and BMD patient myoblasts. If this increase in mRNA levels results in increased protein, this could provide significant therapeutic benefit to both BMD

and DMD patients, in combination with other therapies.

P6: THE ROLE OF TGF-B LIGANDS IN MUSCLE WASTING AND CACHEXIA.

Justin L Chen[1,2], Paul Gregorevic[3], Kelly L Walton[1] and Craig A Harrison[1,2], [1]Prince Henry's Institute of Medical Research, Clayton, VIC, Australia; [2]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia; [3]Baker IDI, Melbourne, VIC, Australia.

Cachexia, a debilitating syndrome characterised by the loss of body mass, fatigue and anorexia, affects up to 80% of patients with advanced cancers and accounts for nearly 30% of cancer-related death. Skeletal muscle wasting is a key feature of cachexia, and its onset greatly reduces quality of life and survival. Strong evidence suggests that members of the transforming growth factor-β (TGF-β) family play a major role in the pathogenesis of the disease. In this study, we showed that intramuscular injections of rAAV6:activin A at vector genome doses of 10^9, 10^{10} and 10^{11} into the tibialis anterior (TA) muscle of C57BL/6 mice caused a 35%, 44% and 42% reduction in muscle mass after 4 weeks (compared with the contralateral control TA), respectively. The decrease in muscle mass was associated with a decrease in fibre cross-sectional area, and activin A over-expression was confirmed by real-time RT-PCR and Western blot. In contrast, intramuscular injections of rAAV6:BMP-7, at the same viral doses, caused a respective 10%, 32% and 54% increase in TA muscle mass. These results indicate an atrophic response with activin A over-expression and a hypertrophic response with BMP-7 over-expression. In addition, as the levels of TGF-β ligands are elevated in many cancers, we have developed a TGF-β antagonist capable of specifically blocking the actions of TGF-β isoforms *in vitro*. Taken together, we have the potential to reverse the effects of muscle wasting and cachexia by inhibiting TGF-β over-expression and, thereby, increasing muscle mass.

P7: NOVEL STRATEGY FOR CELL TYPE SPECIFIC GENE EXPRESSION THROUGH RETROVIRAL TRANSDUCTION OF BONE MARROW STEM CELLS

Jie Yu Chung[1], Zeyad Nasa[1], Amit Joglekar[1] and Frank Alderuccio[1], [1]Autoimmunity Lab, Dept. of Immunology, Central Clinical School, Monash University, AMREP, Commercial Road, Prahran.

Previously our lab has utilised a retroviral vector encoding autoantigen MOG to transduce bone marrow stem cells and promote immune tolerance in recipients. Mice receiving MOG transduced bone marrow were subsequently protected against induction of Experimental Autoimmune Encephalomyelitis (EAE). This showed the potential of utilising gene therapy to treat autoimmune diseases. However because MOG was expressed in all bone marrow derived cells, we were unable to delineate the role of particular cell subsets in tolerance induction. To address this we utilised self-inactivating (SIN) retroviral vectors with tissue specific promoters CD11c (Dendritic cell specific) and lck (T cell specific). Mice receiving bone marrow transduced with CD11c-MOG or lck-MOG did not show the same protection against EAE as previously shown. This may have been due to the relatively low level of gene expression observed when utilising tissue specific SIN retroviral vectors. Therefore there was a need to generate tissue specific gene expression at higher levels to properly investigate the role of individual cell types in our EAE model. Here we describe a novel strategy utilising a Cre-dependent retrovirus to confer cell type specific gene expression at high levels through the bone marrow compartment. Our Cre-dependent retroviral vectors were designed to require Cre mediated DNA

inversion for proper transcription/translation of our genes of interest. Preliminary data show that MOG and GFP marker expression is observed only in the presence of Cre recombinase suggesting that when combined with tissue specific Cre transgenic bone marrow it would be possible to generate tissue specific MOG expression at high levels in vivo.

P8: CONSTRUCTION AND VALIDATION OF A BARCODED LENTIVIRUS STOCK FOR INVESTIGATING SAFETY AND EFFICACY OF GENE THERAPY FOR X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

Claire T Deakin, Samantha L Ginn, Sophia H Liao, and Ian E Alexander, *Gene Therapy Research Unit of the Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, New South Wales, Australia.*

Clinical trials of gene therapy for X-linked severe combined immunodeficiency (SCID-X1) have demonstrated that retroviral gene delivery to relatively small numbers of haematopoietic progenitors can successfully cure a disease phenotype. While cases of leukaemia caused by vector-mediated insertional mutagenesis are well-known, the potential for proliferative pressures on low numbers of haematopoietic progenitors to result in somatic mutations and leukaemogenesis is a further safety concern requiring investigation. For example, it is unknown how features of vector and protocol design may reduce effective cell dose in SCID-X1 gene therapy, and hence be associated with reduced safety. To this end, a barcoded lentiviral plasmid library has been constructed by inserting a short sequence with twelve positions of degeneracy into a lentiviral plasmid construct in which the elongation factor 1α (EF1α) promoter controls expression of the interleukin-2 receptor common gamma chain (γc). The maximum theoretical complexity of this library is over 16.7 million combinations of barcode tags. To initially validate complexity of the plasmid stock, and 20 unique barcode tags have been sequenced, and more comprehensive analysis is being performed using deep sequencing. Lentiviral stocks produced from the barcoded plasmid library have been used to reconstitute γc-deficient mice. The barcode region in genomic DNA of reconstituted T cells isolated from these mice will be amplified and sequenced using the Illumina HiSeq platform. Bioinformatic quantification of the number and relative frequencies of sequenced barcodes will enable estimation of the clonal complexity of the T cell compartment, as well as the relative number of progenitor clones that have contributed to T cell ontogeny.

P9: A KNOCKOUT OF BETA-2-MICROGLOBULIN IN A HUMAN EMBRYONIC STEM CELL BY AAV-MEDIATED GENE TARGETING

Roli K. Hirata[1] Laura Riolobos[1] Yi Li[1] and David W. Russell[1], [1]Department of Medicine, Division of Hematology, University of Washington, Seattle, Washington, USA.

We have previously shown that adeno-associated virus (AAV) gene targeting vectors can be used to genetically engineer human embryonic stem cells. Here we show that in two rounds of AAV-mediated gene targeting of the beta-2-microglobulin (B2M) locus in the human embryonic stem cell line H1, a clone with both alleles containing our insertion vectors was obtained. The clone has been karyotyped and found to be euploid. B2M is essential for the assembly and presentation of HLA class I proteins on the cell surface. HLA class I proteins mediate a self-not self-recognition by the immune system. Our B2M knockout is a first step to engineering a universal donor pluripotent stem cell.

P10: QUANTIFICATION OF TRANSGENE EXPRESSION TARGETED TO DIFFERENT CELL COMPARTMENTS FROM LENTIVIRAL PLASMIDS CONTAINING A FURIN-2A SEQUENCE

Yazad Irani,[1] Sarah Brice,[1] Lauren Mortimer,[1] Helen Brereton,[1] Sonja Klebe,[1] Miriam Keane,[1] Peter Cowan,[2] Doug Coster[1] and Keryn Williams[1], [1]Department of Ophthalmology, Flinders University, Adelaide, Australia, and [2]Immunology Research Centre, St Vincent's Hospital, Melbourne, Australia.

We compared the level of transgene expression from single-gene and double-gene lentiviral plasmid vectors. Double-transgene vectors were constructed by inserting a furin-2A (F2A) self-processing sequence between two transgenes in the vector expression cassette. Analogous single-gene vectors were also constructed. Cloned transgenes included cDNAs for intracellular enhanced yellow fluorescent protein (eYFP) and for two secreted proteins: an anti-rat CD4 antibody fragment (anti-CD4 scFv) and an endostatin-kringle5 fusion protein (EK5). The expression of the transgenes cloned into the double-gene vectors, each transgene upstream or downstream of the F2A sequence, was compared to expression from their single-gene counterparts by transfecting HEK293A or CHO cells with these vectors *in vitro*. Anti-CD4 scFv protein was quantified by flow cytometry on rat thymocytes, EK5 protein by ELISA, and eYFP protein by flow cytometry. Transgenic protein expression from the double-transgene vectors was significantly lower than from their corresponding single transgene counterparts ($p<0.05$). Further, when the transgenic proteins were expressed in *different* cellular compartments, reduced expression of the transgene positioned *downstream* of F2A was observed, compared with expression when it was positioned *upstream* of F2A ($p<0.05$). However, this was not observed when transgenic proteins were expressed in the *same* cellular/extracellular compartment. The implications are that stoichiometric expression of multiple transgenes from a single ORF may occur when all expressed proteins share the same leader sequence, or all lack such a sequence, but not when expressed proteins are expressed in different cell compartments, where it might be preferable to use a cocktail of single transgene vectors.

P11: MOLECULAR SYSTEMS FOR IMPROVING GENE MUTATION CORRECTION IN MUSCLE DISEASE

Robert Kapsa[1,2,3], Gilles Gasser[4], Joe Razal[3], Anita Quigley[1,2], Tanmaya Joshi[5], Simon Moulton[3], Mark J Cook[2], Leone Spiccia[5] David Officer[3] Graeme M Clark[6] and Gordon G Wallace[3], [1]Centre for Clinical Neuroscience and Neurology Research, St Vincent's Hospital, Fitzroy Vic. 3065, [2]The Bionic Ear Institute, East Melbourne, Vic., 3002, [3]Intelligent Polymer Research Institute, University of Wollongong, NSW., 2502, [4]Institute of Inorganic Chemistry, University of Zurich,CH-8057, Switzerland, [5]Department of Chemistry, Monash University, Clayton, Vic., 3168, [6]Hearing and Neuroscience Unit, School of Psychological Science, La Trobe University, Bundoora, Victoria 3086, Australia.

Targeted corrective gene conversion (TCGC) holds much promise as a future therapy for many hereditary diseases in humans, but there still remain significant impediments to effective mutation correction and it is clear that significant work remains to improve TCGC to levels where it can be considered for translation to the clinical setting. Nevertheless, mutation correction frequencies varying between 0.0001% and 40% have been reported using chimeraplasty, oligoplasty, triplex-forming oligonucleotides, and small corrective PCR amplicons. We have

investigated potential mechanisms that impede effective gene correction and potential methods by which to improve the efficiency of dystrophin gene mutation correction in the *mdx* mouse model of Duchenne Muscular Dystrophy (DMD). Arrest of cell cycle using Hydroxyurea and double Thymidine blockade significantly improved TCGC frequency in *mdx* myoblasts at the gene level, but it was clearly evident that transfection reagents rendered TCGC efficiency variable with some, albeit limited expression of the corrected loci. The latter aspect was investigated by adjustment of transfection conditions, resulting in more robust gene correction with better (but still variable) expression of the corrected locus in a proportion of the treated cells, but still affected by the toxic chemistries used to mediate improved gene correction. These studies report new methods and strategies for improvement of TCGC by cell cycle arrest using molecular methods less toxic than transfection reagents or chemical cell cycle inhibitors: Molecular technologies such as PNA chemistry, advanced electromaterials, and CNA motifs that present lesional signals to the DNA metabolic machinery are potential technologies that may impart levels of efficiency that translate more efficiently from gene to protein levels and more readily facilitate consideration towards clinical application.

P12: UTILISATION OF ALBUMIN TO ENHANCE IN VIVO DELIVERY OF SIRNA ACROSS THE VASCULAR ENDOTHELIUM

Shannen Lau[1], Bim Graham[1], Ben J Boyd[2], Colin W Pouton[1] and Paul J White[1], [1]Department of Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Science, Monash University, Parkville, Australia; [2]Department of Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Science, Monash University, Parkville, Australia.

siRNA has great potential as an emerging new therapeutic class, however it is hindered by inefficient delivery *in vivo*. The current study aimed to improve extravasation of siRNA by utilising the transcystosis mechanism of the serum protein albumin to cross vascular endothelial cells. This is achieved by having a well defined, one-to-one siRNA to albumin conjugates. IGF-IR siRNA with 3'-amine-modification was activated with succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate to yield thiol-reactive-siRNA, which was then irreversibly linked to sulfhydryl groups of cysteine residues within serum albumin. *In vitro* conjugation with BSA and mouse serum occurred within 2 minutes and the product was found to be nuclease stable by SDS-PAGE electrophoresis. Conjugates were formed *in situ* with 5 mg/kg intravenous dose over 4 hours in Balb/C mice and resulted in cellular uptake of mesenteric microvasculature and cardiac muscle. 96 hours *in vivo* treatment of thiol-reactive-siRNA (1mg/kg) in Balb/C mice resulted in significant reduction in IGF-IR mRNA to 64.12±4.14% of vehicle-treated organs with continuous endothelia such as left ventricle, whilst the inactivated siRNA had no effect ($n=4$, $P<0.05$). There were no significant changes in mRNA level of kidney samples from animals treated with the thiol-reactive-siRNA ($n=4$, $P<0.05$), whilst the inactivated siRNA showed significant knockdown. However, no significant effects were observed in higher doses of 3 mg/kg thiol-reactive-siRNA. Albumin-conjugated siRNA were therefore observed to extravasate and produce significant gene silencing after intravenous administration.

P13: INVESTIGATING GENE THERAPY RESEARCHERS' ASSESSMENT OF RISKS IN CLINICAL TRIALS

Claire T. Deakin,[1,2] Ian E. Alexander,[1,3] and Ian Kerridge[2,4], [1]Gene Therapy Research Unit, Children's Medical Research Institute and the Children's Hospital at Westmead, [2]Centre for Values, Ethics and the Law in Medicine, Faculty of Medicine, University of Sydney, [3]Discipline of Paediatrics and Child Health, University of Sydney, [4]Department of Haematology, Westmead Hospital.

As clinical trial activity in the gene therapy field increases, the challenge of assessing uncertain risks in research is becoming increasingly apparent. Since there is an inescapable level of risk for any gene therapy trial, decisions must be made with respect to balancing risks in the context of clinical alternatives. When serious adverse events in gene therapy trials attract disproportionate media attention, however, there is a danger that decision-making may in response become increasingly conservative, with the effect of restricting research progress. How researchers make decisions about risk must, therefore, be critically evaluated. We have previously posed the question of whether the gene therapy field is becoming too risk-averse in response to high profile adverse events in gene therapy trials, and are now seeking to investigate how gene therapy researchers make decisions about risks when planning clinical studies. We have constructed a quantitative questionnaire instrument, in which respondents are asked whether they would support the initiation of a clinical trial in different clinical scenarios, when varying characteristics of preclinical knowledge are presented. The influence of various factors on respondents' decision-making will be evaluated, including clinical context and treatment alternatives, age of subjects, and limitations of preclinical models. Gathering empirical data about researchers' decision-making approaches may assist with addressing the challenge of risk assessment in gene therapy research and may facilitate research progress, by both encouraging and informing dialogue about acceptable levels of risk in research.

P14: NANOSTRUCTURED CONDUCTING POLYMER SCAFFOLDS FOR SKELETAL MUSCLE GROWTH

RMI Kapsa[1,2], Razal JM[1], Quigley AF[1,2], Kita M[1,2], Mysore T[1,2], Moulton S[1], Penington AJ[3], Clark GM[4] and Wallace GG[1], [1]ARC Centre of excellence for Electromaterials Science, IPRI, Wollongong NSW 2522, Australia, [2]Centre for Clinical Neuroscience and Neurology Research, St. Vincent's Hospital, Fitzroy, VIC 3065, Australia, [3] Bernard O'Brien Institute for Microsurgery, Fitzroy, Vic., 3065, Australia, [4] Graeme Clark Hearing and Neuroscience Unit, School of Physiological Science, Latrobe University, Bundoora, VIC, Australia.

The 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. We have been investigating the use of nanostructured conducting polymer surfaces for the orientation and electrical stimulation of cells and tissues and have developed a hybrid conducting polymer and carbon nanotube plaform, suitable for *ex vivo* muscle growth, differentiation and electrical stimulation. In this study, Carbon nanotube fibres were laid down in parallel on gold coated mylar. Layers of polypyrrole (doped with pTS) were galvinostatically grown over the carbon nanotube fibre mats to create a nanostructured polypyrrole surface. Myoblasts extracted from 8 week old mice were seeded onto polymer platforms and induced to differentiate into myotubes. Analysis of myofibre differentiation and orientation was then performed. In addition, total RNA was harvested from myotubes that had undergone bipolar electrical stimulation on the platforms 8 hours a day, for 3 days. Whole genome arrays (Codelink) were then used to assess

gene expression changes in the electrically stimulated myotubes.

Platforms were developed to encourage the alignment and differentiation of skeletal muscle stem cells (myoblasts) to reflect the orientation of mature skeletal muscle. Nanostructured platforms were created by orientation of carbon nanotube fibres on a conducting gold mylar surface, over which a layers of conductive polypyrrole were deposited. Human and murine myoblasts could be grown and differentiated on these platforms without the use of cell adhesion molecules. A significant increase in myotube orientation was seen on nanostructured surfaces, i.e. polypyrrole films with an underlying layer of orientated carbon nanotube fibres. This orientation decreased with increasing thickness of the polypyrrole, suggesting a strong influence of the nanostructure on the orientation of myofibres. In addition, a significant number of gene expression changes were detected in myofibres electrically stimulated on the platforms. A number of these genes were associated with muscle differentiation and myoblast fusion, demonstrating that these platforms can be used to influence the differentiation state of skeletal myoblasts through electrical stimulation.

These studies demonstrate that novel hybrid platforms can be used to influence skeletal muscle differentiation *ex vivo*, through electrical stimulation. In addition these platforms can influence muscle fibre orientation in a manner reflecting the *in vivo* architecture of the parent tissue. Such platforms have application for controlling the regeneration of skeletal muscle *in vivo* and for the integration of bionic devices designed to facilitate muscle regeneration and function.

P15: LYMPHOMAGENESIS IN SCID-X1 MICE FOLLOWING IMMUNOLOGICAL RECONSTITUTION CORRELATES WITH PROGENITOR CELL DOSE: DOES REPLICATIVE STRESS PLAY A ROLE?

Samantha L. Ginn[1,2], Sophia H.Y. Liao[1], Min Hu[3], Steven I. Alexander[3] and Ian E. Alexander[1,4]

, [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]Centre for Kidney Research of The Children's Hospital at Westmead and [4]The University of Sydney, Discipline of Paediatrics and Child Health. Westmead, NSW, Australia.

In a previous study we set out to evaluate the efficacy and safety of lentiviral vectors expressing the human γc transgene in a murine model of SCID-X1[1]. We observed lymphoma development in a cohort of mice reconstituted with cells transduced with a construct using the EF1α promoter to drive expression of γc. Extensive analysis revealed that this occurrence could not be attributed to insertional mutagenesis or γc overexpression, illustrating the significant limitations of murine models in the assessment of vector safety. We hypothesised that lymphomagenesis was caused by replicative stress resulting from the transduced progenitors being driven to undergo supraphysiological levels of replication. In the current study, we seek to explore the relationship between progenitor cell dose and the levels of cellular proliferation occurring during lymphopoiesis in the SCID-X1 mouse, and to correlate these with the incidence of lymphoid malignancy. In support of our hypothesis, we have observed the development of T-cell lymphomas in a dose-dependent manner that is statistically different to a cohort of mice receiving a high dose of cells. Interestingly, there also appears to be an inverse correlation between the cell dose received and latency. We are currently elucidating the mechanism of lymphoma

development by assaying for signs of replicative stress during T-cell development.

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

P16: CAN MICE BE TOLERISED TO AAV CAPSID PROTEINS TO ENABLE VECTOR READMINISTRATION?

Grant J. Logan, Maolin Zheng and Ian E. Alexander, Gene Therapy Research Unit, Children's Medical Research Unit and The Children's Hospital at Westmead, 214 Hawkesbury Road, Westmead, NSW 2145.

AAV proviruses and transgene expression in the liver are rapidly lost in the newborn after gene delivery due to cellular proliferation. Re-administration is therefore likely to be an important requirement in this setting for long-term therapeutic benefit after AAV liver transduction. It has been well established, however, that antibody responses to the primary vector hinder secondary vector transduction. We aim to overcome this problem by exploiting the tolerogenic power of the liver to prevent anti-AAV responses. Our strategy is to induce liver expression of AAV serotype 8 VP1 using an AAV vector where codon-optimised transgene expression is driven by a liver-specific promoter (AAV-hAAT-coVP1). This approach has been successfully used to induce tolerance against many other antigens. AAV-hAAT-coVP1 was packaged into serotype 8 capsids and injected into the peritoneal cavity of female Balb/C mice at 1 x 10^{11}vg per injection. Serum was collected at fortnightly intervals until 8 weeks post-injection and assayed for anti-VP1 antibodies by ELISA. Our initial study has shown that, rather than preventing anti-VP1 antibody responses, responses in AAV-hAAT-coVP1 injected mice were increased when compared to control mice injected with vector encoding *ornithine transcarbamylase*. Also, antibody affinity for VP1 in AAV-hAAT-coVP1 injected animals was higher than in control groups. Finally, liver transduction of a second vector (AAV-hAAT-GFP) was significantly impaired in both groups when compared with transduction in a naïve control group of mice. We hypothesise that tolerance induction to AAV capsid molecules in this model may require higher levels of VP1 transgene expression to be effective.

P17: ALTERNATIVE SPLICING OF LAMIN A LEADS TO AGE-DEPENDENT ACCUMULATION OF PROGERIN TRANSCRIPT IN NORMAL HUMAN MUSCLE AND SPORADIC INCLUSION BODY MYOSITIS (S-IBM)

Yue-Bei Luo[1], Vicki Fabian[2], Russell Johnson[1], Sue Fletcher[1], Steve Wilton[1], Frank Mastaglia[1], [1]Centre for Neuromuscular and Neurological Disorders, Australian Neuro-Muscular Research Institute,University of Western Australia, Perth, Australia; [2]Section of Neuropathology, Department of Anatomical Pathology, Royal Perth Hospital, Perth, Australia.

Mutations in the Lamin A gene (*LMNA*) are known to cause Hutchinson-Gilford progeria syndrome. In HGPS a silent mutation at codon 608 activates an alternative splice site in exon 11 resulting in an internally truncated mRNA (Δ150 bases) and accumulation of a shorter lamin A isoform, named progerin. Expression of progerin at low levels also occurs normally in skin, liver and heart but has not been investigated in skeletal muscle. In this study we investigated whether there is age-dependent accumulation of progerin transcript in muscle biopsy from 13 healthy adults (37-71 years of age). RNA was extracted from biopsy samples and RT-PCR was performed. A product 150bp smaller than lamin A, which was confirmed to be progerin by DNA sequencing, was present in 7/13 samples. All individuals over 60

years had a progerin band with the youngest being 44 years. Considering that there is acceleration of the aging process in s-IBM muscle we also investigated 7 s-IBM cases (aged 57-84 years). Progerin amplicons were present in all 7 biopsies and the strongest progerin bands of the two groups were found in two of the s-IBM samples. These preliminary findings indicate that there is accumulation of progerin in skeletal muscle with aging and suggest that progerin expression may be increased in s-IBM. It has yet to be established that this level of progerin expression is associated with any pathological changes in aging tissue or s-IBM, but offers a potential target for splice-switching oligomers to suppress this low level of aberrant mis-splicing.

P18: LOW TOXIC NON-MYELOABLATIVE REGIMEN AS PRECONDITIONING FOR INDUCTION OF IMMUNE TOLERANCE USING GENE THERAPY

Nasa Z[1], Chung J[1], Joglekar A[1], Chan J[2], Toh B[2], Alderuccio F[1], [1]Department of immunology, Monash central clinical school, Monash University, AMREP, Prahran, Victoria, Australia; [2]Department of Medicine, Monash university, Clayton, Victoria, Australia.

Autoimmune diseases are characterized by a chronic adaptive immune response that targets self-antigens and leads to clinical pathology. Treatments of autoimmune diseases are often non-specific and do not address the cause, but aim to reduce symptoms. Multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) are autoimmune diseases of the central nervous system. We have previously shown that transplantation of bone marrow (BM) cells transduced with retrovirus encoding the myelin autoantigen myelin oligodendrocyte glycoprotein (MOG), into total body irradiated mice can prevent the induction of EAE. This work has shown the potential of utilising BM gene therapy to cure autoimmune diseases; however the undesirable side effects of total body irradiation remain an obstacle for translation to the clinic. In this study, we have investigated the use of Treosulfan as a less toxic preconditioning regime and assessed the induction of tolerance and disease susceptibility to provide potential clinical feasibility assessment. Transfer of BM expressing MOG into partially myeloablated and non myeloablated mice using Treosulfan resulted in molecular chimerism and robust protection from the EAE. Moreover, in clinically relevant scenario of established EAE we could also promote immune tolerance and long-lasting disease resistance using a combination of corticosteroid treatment to induce initial remission, followed by Treosulfan at non myeloablative dose and the transfer of transduced BM to maintain long-term remission. Mice remained resistant to EAE even upon subsequent rechallenge with MOG-peptide. These results suggest that less toxic and more clinically applicable preconditioning regimes can be utilised to promote immune tolerance for the prevention and treatment of established autoimmune diseases.

P19: ENHANCING HISTONE-MEDIATED GENE DELIVERY THROUGH INCREASED NUCLEAR TARGETING

Michael Nastasie[1], David A. Jans[1], Helmut Thissen[2] and Kylie M. Wagstaff[1], [1]Nuclear Signalling Laboratory, Dept. of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; [2]Molecular and Health Technologies, CSIRO, Clayton, Victoria, Australia.

Whilst existing viral-based gene therapy approaches are highly efficient, they are associated with significant safety concerns

including immunogenicity and residual pathogenicity, as well as being limited in their DNA carrying capacity and by the cost of vector production. Non-viral gene therapy vectors, in contrast, whilst safe, are limited most notably by inefficient transport of DNA to the nucleus. Histones are an attractive possibility in this context, being innately capable of binding DNA, entering cells by protein transduction, and transporting it to the nucleus with high efficiency to be expressed. In this study we focussed on histones H3 and H4, engineering the optimised Simian Virus SV40 large tumour antigen (T-ag) nuclear localisation signal (NLS) into both to enhance nuclear targeting. We have shown that the bacterially expressed histones retain their DNA binding ability, with the T-ag NLS enhancing nuclear targeting of the histones significantly in mammalian cells. The nuclear targeting optimised histones H3 and H4 are great prospects for use in therapeutic gene delivery, with experiments now underway to test these newly engineered vectors for solid-phase DNA delivery in a novel reverse transfection cell microarray system for high-throughput DNA delivery to distinct cell populations. The ultimate aim is to develop novel histone delivery vectors for cancer cell specific gene therapy.

P20: MICRORNAS AS TOOLS TO TARGET BONE MARROW MEDIATED TUMOUR ANGIOGENESIS

P.N. Plummer[1], R. Taft[2], J. Mattick[2], N. McMillan[3], A. Swarbrick[4], R. Brink[4], V. Mittal[5], A.S. Mellick[1], [1]Host Response to Cancer Lab, School of Medical Science, Griffith University, Gold Coast, QLD, [2]Institute of Molecular Biosciences, University of Queensland, Brisbane, QLD, [3]Diamantina Institute, University of Queensland, Brisbane, QLD, [4]Garvan Institute of Medical Research, Sydney, NSW, [5]Medical College, Weill Cornell University, New York, NY, USA.

Targeting the process of tumour vascularization is a key aspect of modern anticancer therapy. However, the cellular and molecular mechanisms by which tumours enroll vasculature remains relatively undefined. Furthermore, the role of host inflammatory cells, and bone marrow derived (BMD) cells in this process, despite extensive research, remains controversial. BMD endothelial progenitor cells (EPCs) have been identified by our lab and others as being critical for the angiogenic switch[1-3]. However, because different markers are used to track these cells in the bone marrow, blood and tumour-stroma, controversy exists as to whether the same cell is truly being followed *in vivo*. Using a lentiviral and transgenic mouse model we have the Inhibitor of DNA Binding 1 (Id1) can be used to mark and track EPCs from the BM to their incorporation as part of the tumour vasculature[1]. This work also demonstrated that Id1 can be used to target EPCs and suppress tumour angiogenesis. Small RNA Illumina analysis has identified small non-coding RNAs in particular microRNAs (miRNA) which distinguish tumour vasculature from lung vasculature; as well as specific changes in BM lineages following tumour challenge. Fluorescent miRNA In-situ Hybridization has also been used to demonstrate localization of several of these microRNAs to tumour vasculature and EPCs.

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P21: AVOIDING FALSE POSITIVES WITH IN VIVO RNAI

Glen Reid, Asbestos Diseases Research Institute, University of Sydney, Concord, NSW, Australia

RNAi has become widely used to analyse gene function but several difficulties with its use *in vivo* remain, due to inefficient delivery and non-specific effects of siRNAs in animal models. Specific detection of mRNA cleavage by 5'RACE is the only method to confirm the knockdown of mRNA by RNA interference, but is rarely reported for *in vivo* studies. We have combined 5'-RNA-Linker Mediated RACE with real-time PCR using a molecular beacon specific for the site of siRNA-mediated cleavage to develop a rapid and specific method termed MBRACE. Following siRNA transfection *in vitro*, *ApoB*- and *RRM1*-specific MBRACE reactions detected the predicted mRNA cleavage products. Specific cleavage was detected in the livers of mice following administration of *ApoB*-specific siRNA, even in cases where mRNA knockdown was less than 10 %. In contrast, apparent siRNA-mediated knockdown of *RRM1* in tumours was dependent on the qPCR primers. Two siRNAs with equivalent activity *in vitro* were administered to A549 xenografts via intratumoral injection. In each case, MBRACE results were negative and apparent knockdown of *RRM1* mRNA was observed only when the qPCR primers flanked the siRNA cleavage site. This suggests that siRNA injected into tumours may be co-purified with total RNA and interfere with downstream analysis, and that primers flanking the siRNA target site should be avoided when measuring knockdown of target mRNA *in vivo*. Using the MBRACE method to detect mRNA cleavage is a simple way to corroborate knockdown studies and avoid false positives following siRNA use *in vivo*.

P22: NEUROTROPHIN GENE THERAPY FOR AUDITORY NERVE SURVIVAL AND DIRECTED FIBRE REGROWTH AFTER HEARING LOSS

Rachael T. Richardson[1,2], Patrick Atkinson[1,2], Tian Tu[1,2], Andrew Wise[1,2], Brianna Flynn[1], Cliff Hume[3], Stephen O'Leary[1,2] and Rob Shepherd[1,2], [1] The Bionic Ear Institute, [2]The University of Melbourne, Department of Otolaryngology, [3] The University of Washington, Department of Head and Neck Surgery

A cochlear implant helps people with severe-profound deafness to hear speech. It relies on spiral ganglion neurons (SGNs) to transmit signals from the electrodes to the brain. However, SGNs degenerate after deafness due to loss of neurotrophins normally secreted by the cochlear sensory epithelium. Injecting neurotrophins into the cochlea prevents SGNs degeneration but provides no directional guidance to resprouting fibres. This research investigates the applicability of adenoviral gene therapy in the cochlea to create a localised neurotrophin source for SGN survival and directional SGN fibre resprouting following deafness in guinea pigs. We compared localised versus ubiquitous cochlear gene expression on SGN survival and fibre resprouting, investigated the effect of prolonged deafness on the efficacy of gene transfer and examined the longevity of the neural responses to gene expression. Localised neurotrophin gene expression in the sensory epithelium (by injection into the scala media compartment of the cochlea) resulted in 1.8-fold greater SGN survival compared to ubiquitous gene expression (from injection into the scala tympani compartment) ($p<0.05$). Furthermore, there was directional SGN fibre growth towards areas of localised neurotrophin gene expression. While the efficacy of gene therapy diminished as the period between deafness and gene therapy increased, our research indicated that the effects of neurotrophin gene therapy were long-term with significant SGN survival observed at 11 weeks post-treatment ($p<0.05$). We conclude that gene therapy can create a localized neurotrophin source to maintain SGN survival and control SGN fibre regeneration after deafness and help improve sound perception with a cochlear implant.

P23: OVERCOMING OBSTACLES TO CONSTRUCTING RAAV VECTORS FOR PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 3

Susan M. Siew[1], Ian E. Alexander[1,2], [1]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Australia; [2]Discipline of Paediatrics and Child Health, University of Sydney, Australia.

Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3), an inherited liver disease caused by mutation of the ABCB4 gene, is characterised by cholestasis and progressive biliary cirrhosis, eventually requiring liver transplantation. Lack of efficacious alternative treatments, a well characterised mouse model for pre-clinical investigation, and ability to target gene transfer to hepatocytes using adeno-associated virus (rAAV), make PFIC3 an attractive candidate for liver directed gene therapy. ABCB4 encodes a phospholipid floppase on hepatocyte canalicular membranes necessary for biliary phospholipid excretion. In this study, we aim to develop and characterise a rAAV vector encoding human ABCB4 cDNA using a knockout mouse model of PFIC3. Recombination deficient *Escherichia coli* strains are commonly used for faithful propagation of recombinant DNA in cloning procedures. In constructing this vector, we observed markedly slower growth and strong selection against recombination deficient clones harbouring intact ABCB4 cDNA. Severe growth impairment occurred when ABCB4 was placed downstream of bacterial LacZ promoter, but could be partially improved by using SOC growth medium containing glucose, which suppresses LacZ promoter activity. We postulate that expression of toxic gene product was detrimental to host viability. In developing a lentiviral strategy for PFIC3, a Dutch group observed ABCB4 mediated alterations to lentiviral envelop phospholipid composition that resulted in insufficiently low viral titres and reduced infectivity. Though utilizing encapsidated rAAV should surmount such difficulty, we speculate that bacterial host toxicity could also be due to altered membrane lipid composition. Overcoming these obstacles is inherent in developing a viable gene therapy vector for PFIC3.

P24: MIR-200B PREVENTS TGFβ1-INDUCED EMT AND FIBROSIS IN KIDNEY PROXIMAL TUBULAR CELLS

Owen Tang, XinMing Chen, Carol Pollock, Renal Medicine, Kolling Institute, University of Sydney, Sydney, New South Wales, Australia.

Aim: To investigate the role of miR-200b in mediating molecular and cellular changes those modify renal interstitial fibrosis in the presence of transforming growth factor beta (TGFβ).

Background: MicroRNAs comprise a novel class of endogenous small noncoding RNA that control the expression of target genes. Recent reports suggest that miR-200b prevents EMT in cancer cell by targeting the E-box binding transcription factors Zeb1 and Zeb2. Hence this study was designed to assess the effect of miR-200b on EMT in renal tubular cells.

Methods: Immortalised human proximal tubular cells (HK2) were transfected with 25nM miR-200b before exposure to TGFβ1 (0.5ng/mL) for 48 hours. mRNA and protein expression of E-Cadherin, Zeb1, Zeb2 and fibronectin were measured using qPCR and western blot analysis.

Results: Cells transfected with miR-200b did not develop EMT and retained their epithelial cell characteristics when exposed to TGFβ1. miR-200b significantly increased E-Cadherin mRNA expression by 20 fold ($p<0.001$) and reduced fibronectin mRNA expression by 2 fold compared to non-specific miRNA ($p<0.01$). mRNA of Zeb1 and 2 was not

changed significantly by miR-200b. However, a significant reduction in Zeb1 protein expression was evident by western blot.

Conclusions: Data suggest that miR-200b suppressed the E-box binding transcription factors Zeb1 and/or Zeb2 to maintain epithelial phenotype to reduce ECM production in human tubular cells exposed to $TGF\beta_1$. However the mechanism of how miR-200b works *in vivo* still needs to be determined by experiments which require sophisticated delivery methods.

P25: ENGINEERING INDUCED PLURIPOTENT STEM CELLS (IPSC) REPORTER LINES USING ADENO-ASSOCIATED VIRUS (AAV) MEDIATED HOMOLOGOUS RECOMBINATION

Maolin Zheng[1], Grant J. Logan[1], Christine M. Smyth[1], Joshua B. Studdert[2], Patrick P. L. Tam[2] and Ian E. Alexander[1], [1]Gene Therapy Research Unit, Children's Medical Research Unit and The Children's Hospital at Westmead, [2]Embryology Unit, Children's Medical Research Unit, 214 Hawkesbury Road, Westmead, NSW 2145.

Reprogramming somatic cells to pluripotency by the enforced expression of key transcriptional factors provides the opportunity to generate patient-specific stem cells. These so-called induced pluripotent stem cells (iPSC) can be subsequently differentiated into cell types that are normally difficult to access, including neurons and hepatocytes, and thereby provide a renewable source of cells for disease study and cellular therapies. However, a current challenge is to develop protocols that faithfully recapitulate the complex signals during organogenesis to differentiate stem cells into desired cell types. Progress in this area would be facilitated by reporter cell lines where expression of fluorescent molecules would reveal important milestones of cell differentiation. Advantages of this approach are the provision of a visual indicator of cellular differentiation and the ability to sort and analyse cellular subpopulations. As an initial step, we reprogrammed human foreskin fibroblasts into iPSC using a lentivirus vector encoding Oct4, Sox2, Klf4 and cMyc (Sommer et al. 2009 *Stem Cells*). Consistent with an iPSC phenotype, select clones are found to express Nanog, Rex1, Oct4, Sox2, ABDG-2 and DNMT3B. We are currently engineering these clones into reporter lines using AAV-mediated homologous recombination, a process shown to be highly efficient in many cell types including human fibroblasts and iPSC (Khan *et al*. 2010 Molecular Therapy). The albumin locus has been targeted by inserting cDNA encoding GFP downstream of the promoter. Clones are currently being screened to identify bona fide recombination events to establish the desired reporter cells for use in endoderm differentiation.