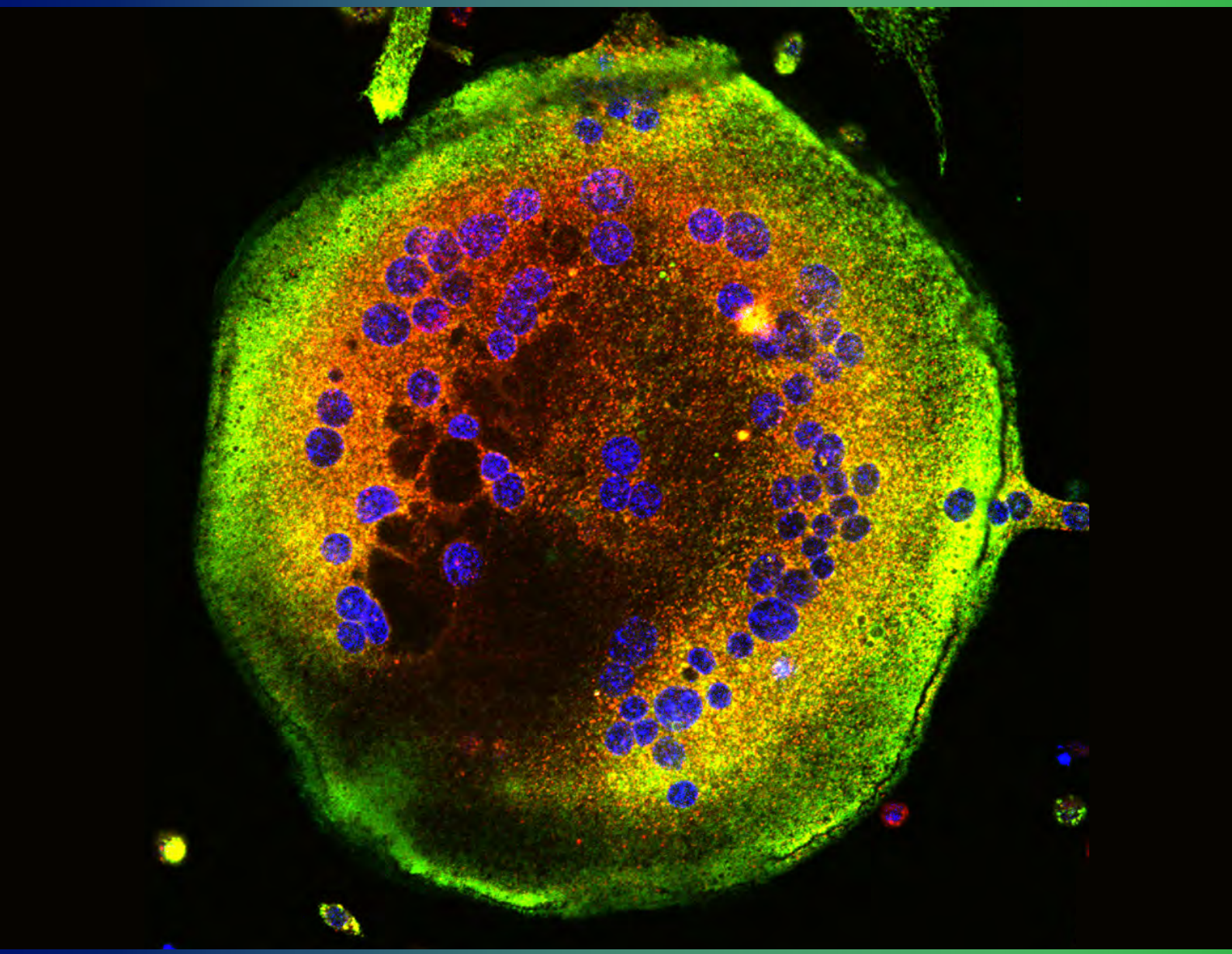
CBSM

2019 Combined Biological Sciences Meeting



The University Club
30th August 2019

Promoting biological science in Western Australia

POSTER ABSTRACTS

SECTION	TOPIC	AWARD SPONSOR
A	Biochemistry & Molecular Biology	ASBMB
B	Health Sciences	CBSM
C	Senior Research Presentations	John Morris Scientific
D	Plant Science	CBSM
E	Cell & Developmental Biology	ANZSCDB
F	Immunology	ASI
G	Neuromuscular & Neuroscience	Perron Institute
H	Genetics	HGSA
I	Microbiology	ASM
J	~Omics	Murdoch University

Kes1-dependent Sphingolipid Signaling Inhibits Ire1 Clustering to Attenuate the Unfolded Protein Response.

Mark Bieniawski[1], Kofi Stevens[1], Rob Steuart[1], Vytas Bankaitis[2] and Carl Mousley[1],

[1] School of Pharmacy and Biomedical Sciences & CHIRI, Curtin University, Australia. [2] Texas A & M Health Science Center, Department of Molecular and Cellular Medicine, College Station, U.S.A

Oxysterol-binding proteins (OSBPs) constitute a large and highly conserved family of proteins in Eukaryotes and are generally involved in transporting membrane lipids between organelles. Thus, OSBPs are crucial for maintaining lipid homeostasis. The Eukaryotic model *Saccharomyces cerevisiae* encodes for 7 OSBPs, each with unique organellar and/or lipid-binding specificities. Kes1/Osh4 (mammalian ORP1-S) is a small OSBP which harbours unique regulatory capabilities in cells not seen in other yeast OSBPs. Additional to its primary activity of transporting sterol between the *trans*-Golgi network and the endoplasmic reticulum (ER), Kes1 activity interestingly presents as a potent antagonist of essential pathways such as vesicular trafficking and TOR-dependent cell growth. Here we identify a novel Kes1-dependent mechanism which negatively regulates the onset and amplitude of the unfolded protein response (UPR). The UPR is activated during times of ER stress, either through accumulation of misfolded proteins, or though build-up of secretory cargo. During the UPR, secretory activities from the ER are reduced and the luminal environment is modulated until homeostasis is restored. The master activator of the UPR is the ER transmembrane protein Ire1, which upon detection of ER stress conditions activates a potent transcription factor for UPR genes. This activity is further optimised when Ire1 forms large clusters across the ER membrane. Using fluorescence microscopy, we show that Kes1 activity antagonises UPR activation by inhibiting the formation of Ire1 clusters. Furthermore, we identify that Kes1-dependent sphingolipid metabolism is a major contributor to this effect. By treating cells with a ceramide precursor phytosphingosine, Ire1 does not efficiently form clusters. Due to the major role of Kes1 in regulating secretory activity, we propose that Kes1 activity is modulated in cells to regulate the intensity of the UPR to levels appropriate for experienced secretory stress conditions.

Exploring a Role for Sss1p in Modulating ER Translocon Gating

Christopher Witham[1,2], Kofi Stevens[1,2], Robert Steuart[2] and Carl Mousley[1],

[1] School of Pharmacy and Biomedical Sciences, Curtin University[2] Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University

Translocation at the Endoplasmic reticulum (ER) is an important process in protein biogenesis via facilitating the transport of secretory and integral membrane proteins to the ER lumen. The passage of these proteins is critical in their maturation as the ER provides an environment that facilitates post-translational modifications. Two modes of translocation exist for the varying polypeptides that proceed via the ER, described as SRP dependent and SRP independent translocation. Centric to these two pathways is a heterotrimeric protein complex known as the Sec61 complex. Sec61 is composed of two essential subunits, Sec61p/Sec61α and Sss1p/Sec61γ and one non-essential subunit Sbh1p/Sec61β, which together form a stable complex with an aqueous pore that facilitates the movement of a nascent polypeptide. Of the essential subunits, the Sss1p subunit is the least characterised being currently described as a “clamp” that acts to stabilise the Sec61p subunit. Through employing the eukaryotic model organism, S*accharomyces cerevisiae*, we found the highly conserved extreme C-terminus of Sss1p to be essential for translocon function. Two mutations within this region, coined *sss1-6* and *sss1-7*, that demonstrate temperature sensitivity (TS) at 37°C have been characterised; finding that ER stress is induced while complex integrity is still maintained in a manner that is still proficient for ER translocation. Furthermore, we have been successful in supressing this TS phenotype through mutations in the *SEC61* gene that were found to lie within key regions for translocon gating; of these mutations some have been previously described in modulating such a role. Several gating assays have been utilised to collectively suggest that the TS Sss1p mutants are destabilising the closed conformation of this channel, whereas the suppressive Sec61p mutants appear to alleviate this outcome. Hence, we propose a role for the extreme C-terminus of Sss1p in modulating translocon gating.

A3

TransloControl: Sss1p/Sec61γ a Novel Quantity Control Substrate

Christopher Witham[1,2], Kofi Stevens[1,2], Robert Steuart[2] and Carl Mousley[1],

[1] School of Pharmacy and Biomedical Sciences, Curtin University, [2] Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University

The endoplasmic reticulum (ER) is a multifunctional organelle essential for its role in the biogenesis of secretory and integral membrane proteins. These proteins and the roles played by the ER are critical in cellular physiology and can be implicated in various pathological outcomes. Passage of a nascent polypeptide into the ER is facilitated via the heterotrimeric protein complex known as the Sec61 translocon. Sec61 comprises of two essential subunits, Sec61p/Sec61α and Sss1p/Sec61γ and the non-essential Sbh1p/Sec61β subunit. Together these proteins interact to form an aqueous pore that conducts protein movement to the ER lumen while maintaining ER membrane integrity. Sss1p, the lesser understood of the essential subunits, is often described as having a role in stabilising the translocon complex. Structural analysis of Sss1p identified a heptapeptide (K_{69}LIHIPI$_{75}$) within the extreme C-terminus that is absolutely conserved in all eukaryotes studied to date. As such this study utilised S*accharomyces cerevisiae* as a eukaryotic model when investigating the functionality of this region. Alanine scanning mutagenesis was performed, producing four double alanine mutants. Of those mutants two, designated *sss1-3* ($I_{68}K_{69}$) and *sss1-6* ($P_{74}I_{75}$), were found to elevate Sss1 protein levels. Cycloheximide (chx) chase was performed to investigate this phenomenon finding Sss1p level to stabilise in these mutants; an outcome that was similarly reflected upon investigation of *sss1* within *hrd1Δ* and *doa10Δ* mutants, components in ER associated degradation (ERAD). The degradation of sss1p was also examined under the absence of the proteins binding partners. In essence, we have demonstrated Sss1p to be a short-lived integral membrane protein and identified a highly conserved region which encodes it's degron. We also suggest that Hrd1p and Doa10p recognise different regions of the K_{69}LIHIPI$_{75}$ degron, a potential avenue for future work as we begin to elucidate the essential nature of the Sss1p/Sec61γ subunit.

A4

Antisense Oligonucleotide Mediated Splice Modulation to Improve CFTR Function of Intron 9 5T Polymorphism

[1,2,6] Kelly Martinovich, [1,2,3,4] Anthony Kicic, [5,6] Sue Fletcher, [5,6] Stephen D Wilton, [1,2,3,4] Stephen M Stick on behalf of [1,3,7] AREST CF and [1, 2, 3, 8] WAERP

[1]UWA. [2]Telethon Kids Institute. [3]Dept. of Respiratory, PCH. [4]School of Public Health, Curtin Uni. [5]The Perron Institute, UWA. [6]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch Uni. [7]Murdoch Children's Research Institute. [8]St. John of God hospital, Subiaco.

Introduction: Over 2000 mutations in the Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene causes cystic fibrosis (CF) with variable clinical phenotypes. The length of the poly T tract in intron 9 influences exon 10 selection and can manifest as mild or severe disease depending on other *CFTR* mutations. Manipulation of *CFTR* pre-RNA splicing using antisense oligonucleotides (AOs) is a potential therapy for selected CF causing mutations. We aim to develop splice modulating AOs to rescue CFTR function in CF patients that carry the shorter 5T polymorphism in intron 9. **Methods:** Multiple AOs targeting *CFTR* intron 9 and the flanking exons; 9 and 11 were designed and initially optimised using 2'-O-Methyl modified bases on a phosphorothioate backbone (2OMe) and transfected into primary airway epithelial cells from a child with p.508del/Arg117His;5T CF. After 48 hours RNA was collected, and PCR was used to determine the ratio of altered transcript compared to full-length product. **Results:** Of the 32 2OMe AOs tested for exon 10 inclusion, none reduced the intron 9 5T induced exon 10 skipping. Of the 6 AOs designed to skip Exon 11, the highest efficiency was 22% from the intron 9 5T allele. **Conclusion:** *CFTR* exons 9 and 11 can be skipped mRNA using very low concentrations of AOs. We propose that skipping the exons flanking exon 10 (9 and/or 11) on the *CFTR* 5T allele could improve CFTR function in CF patients carrying selected mutations, either alone or in combination with current therapeutics. Supported by: USCF; NHMRC; CFWA.

A5

Is OLIG3 Expressed in Glioblastoma and a Possible Target of the Novel Drug CT-179?

Stacey Cilemanoff[1,2], Jeffreena Miranda[2], Naomi Alexander[2], Terrance Johns[2,3]

[1]School of Pharmacy and Biomedical Sciences, Curtin University, [2] Telethon Kids Cancer Centre, Telethon Kids Institute, [3]The University of Western Australia

Glioblastoma (GBM) is the most prevalent and the most aggressive malignant primary brain cancer. GBM arises from mutations in neural stem cells and neural and glial progenitor cells. One molecular marker of GBM, is the over-expression of OLIG2; a homodimerized transcription factor that normally enables differentiation and growth of those progenitor cells. But in GBM, it contributes to its proliferative, invasive and treatment resistant nature. This led to the creation of CT-179, which alters OLIG2's conformation, preventing its function, to result in tumour regression as shown in zebrafish and mouse models. The problem lies in the lack of research regarding OLIG3's role in GBM, OLIG2 function and CT-179 interaction, as it's genetically 94.6% similar to OLIG2. Therefore, it is our hypothesis that OLIG3 is expressed in patient-derived GBM cell lines where it dimerizes with OLIG2 and is a target of the drug CT-179. To investigate this, the following techniques will be conducted on patient derived GBM cell lines with varied OLIG2 expression: immunoblotting to demonstrate OLIG3 protein expression, PCR to demonstrate OLIG3 mRNA expression, cellular thermal shift assay to demonstrate if CT-179 binds with OLIG3 and co-immunoprecipitation to demonstrate if OLIG3 and OLIG2 form a heterodimer transcription factor complex. Through immunoblotting, we have found that like OLIG2, the expression of OLIG3 differs between patient-derived GBM cell lines. The expression levels between OLIG2 and OLIG3 appear to be the inverse of each other but only in very high and very low OLIG2 expressing cell lines. This research will contribute to the understanding of the drivers of GBM tumorigenesis and what proteins CT-179 targets. It will also help develop more effective treatments for GBM that could be translated into the clinic and increase patient survival. Most importantly, it will fill the gap in knowledge regarding OLIG3's role in GBM.

A6

In vitro Cellular Uptake and Neuroprotective Efficacy of Poly-arginine-18 (R18) and Poly-ornithine-18 (O18) Peptides: Critical Role of Arginine Guanidinium Head Groups for Neuroprotection.

Gabriella MacDougall[1,2,3], Ryan S. Anderton[2,3,4], Eden Ouliel[2,3], Junjie Gao[2,5,6], Sharon L. Redmond[7,8], Neville W. Knuckey[1,2], and Bruno P. Meloni[1,2,4]

[1] Department of Neurosurgery, Sir Charles Gairdner Hospital, QEII Medical Centre [2] Perron Institute for Neurological and Translational Science [3] School of Heath Sciences, The University Notre Dame [4] Centre for Neuromuscular and Neurological Disorders, UWA [5] Centre for Orthopaedic Research, Faculty of Health and Medical Sciences, UWA [6] Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China [7] Ear Science Institute Australia [8] Ear Sciences Centre, School of Surgery, UWA

Introduction. We have previously demonstrated that CARPs, in particular poly-arginine-18 (R18; 18-mer of arginine), exhibit potent neuroprotective properties in both *in vitro* and *in vivo* neuronal injury models. The neuroprotective properties of CARPs and their ability to traverse cell membranes is thought to be a direct result of arginine residues by virtue of their positive charge and guanidinium head group. **Problem Statement**. This study examined the importance of arginine guanidinium head groups in R18 for peptide cellular uptake, localization and neuroprotection. This was achieved by using poly-ornithine-18 (O18; 18-mer of ornithine) as a control, which is structurally identical to R18, but possesses amino head groups rather than guanidino head groups. **Procedures.** Epifluorescence and confocal fluorescence microscopy was used to examine cellular uptake and localization of FITC-conjugated R18 and O18 in primary rat cortical neurons and SH-SY5Y human neuroblastoma cells. An *in vitro* cortical neuronal glutamic acid excitotoxicity model was used to compare the effectiveness of R18 and O18 to inhibit cell death and intracellular calcium influx, as well as caspase and calpain activation. **Results.** Fluorescence imaging studies revealed cellular uptake of both FITC-R18 and FITC-O18 in neuronal and SH-SY5Y cells, however intracellular localization of the peptides differed in neurons. Following glutamic acid excitotoxicity, only R18 was neuroprotective, prevented caspases and calpain activation, and was more effective at reducing neuronal intracellular calcium influx. **Conclusions.** For long chain cationic polyarginine peptides (e.g. 18-mers), the guanidinium head groups provided by arginine residues are essential for neuroprotection but are not required for entry into neurons.

Evaluation of Novel Antisense Oligonucleotide-mediated Splice Modulation for Duchenne Muscular Dystrophy

Suxiang Chen[1,2], Bao T. Le[1,2], Madhuri Chakravarthy[1,2], Kamal Rahimizadeh[1], Tamer R. Kosbar[1] & Rakesh N. Veedu[1,2]

[1]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Australia, [2]Perron Institute for Neurological and Translational Science, Australia.

Introduction. Chemically modified antisense oligonucleotide (AO)-mediated exon skipping has been established as a successful strategy for the treatment of Duchenne muscular dystrophy (DMD). Eteplirsen, a fully phosphorodiamidate morpholino (PMO)-modified AO drug has been approved by the US FDA for the treatment of DMD while drisapersen, a fully 2'-O-methyl (2'-OMe) phosphorothioate (PS)-modified AO candidate was rejected based on safety issues. **Problem Statement**. PMO oligos showed excellent safety profile, however, PMO is not compatible with other chemistries to synthesize mixmer AOs which make it more expensive and in addition, large scale production of PMOs is challenging due to difficult synthesis chemistry unlike 2'-OMe-PS chemistry. To overcome this, we envisioned the development of novel morpholino nucleic acid (MNA) to synthesise mixmer oligonucleotides in combination with 2'-OMe-PS nucleotides. **Procedures/Data/Observations**. In our study, we explored the scope of MNA/2'-OMe-PS mixmer AO (AO1) together with other modified AO candidates such as 2'-deoxy-2'-fluoro (2'-F)/2'-OMe-PS mixmers (AO2, AO3, AO4) and 2'-F/locked nucleic acid (LNA)-PS mixmers (AO5, AO6, AO7) to induce exon-23 skipping in mdx myotubes in vitro. **Results**. The MNA/2'-OMe-PS mixmer, AO1 achieved a comparable exon-23 skipping to its fully 2'-OMe-PS AO control (~60%) at 400 nanomolar concentration. Furthermore, all of the 2'-F/2'-OMe-PS mixmers (AO2, AO3, AO4) and 2'-F/LNA-PS mixmers (AO5, AO6, AO7) induced higher exon-23 skipping (14%, 31%, 24%, 33%, 33%, 18%) in comparison with their fully 2'-OMe-PS AO control (13%) at extremely low concentration (2.5 nanomolar). **Conclusions**. Based on our preliminary results, AOs containing mixmer chemistries could be useful in achieving efficient splice modulation in cells.

Is the Presence of High Risk Human Papilloma Virus in Placental Tissue Associated with an Increased Risk of Pre-eclampsia in a Cohort of Western Australian Pregnancies?

Lisa Motteram[1], Julie Sartori[1], David Coall[1], Megan Lloyd[1],
[1]Edith Cowan University, Western Australia.

Human papillomavirus (HPV) is the most prevalent sexually transmitted disease in the world and a common causative factor for cervical cancer. Recent evidence suggests that high-risk HPV infects placental tissue and can undergo complete replication and virion production. There is evidence to suggest that HPV can alter trophoblastic function, and subsequently have deleterious effects on adhesion, implantation and decidualisation. To elucidate the relationship between high-risk strains of HPV (16 & 18) in placental tissue and the disease state pre-eclampsia, immunohistochemistry and PCR will be performed on an existing biobank of placental tissue from the Placenta Project cohort. This project will investigate the presence of the high-risk HPV E6 protein in human placental tissue and will interrogate the correlation between infection with high-risk HPV and early onset pre-eclampsia, in a Western Australian cohort.

Placental Alterations and Fetal Growth Restriction: a Dexamethasone-induced Intrauterine Growth Restriction (IUGR) Rat Model

Yutthapong Tongpob[1,2], Shushan Xia[3], Andrew Mehnert[3,4], Alexander Joos[3], Kirk Feindel[3], Caitlin Wyrwoll[1]
[1] School of Human Sciences, The University of Western Australia (UWA), Australia, [2] Faculty of Medical Sciences, Naresuan University, Thailand, [3] Centre for Microscopy, Characterisation and Analysis, UWA, Australia, [4] National Imaging Facility, UWA, Australia.

A key driver of intrauterine growth restriction (IUGR) is placental insufficiency, including inadequate development of placental vasculature. However, current technical challenges encumber reliable and valid evaluation of *in-vivo* placental function. MRI can measure relaxation time (T2*), which correlates with oxygenation via alterations in blood haemoglobin saturation. *In-vivo* Placental T2* studies to date have shown conflicting results, partly due to technical and analytical limitations confining to only 2D regions of interest (ROI). This study utilised a 3D-MRI approach for serial evaluation of *in-vivo* placental function in a rat model of IUGR. Time-mated rats were treated with either vehicle (Veh) or dexamethasone (Dex; 0.5µg/ml) from embryonic day (E)13 onwards. Serial scans at E15, E18, and E21 were conducted using a 9.4T MRI *in-vivo*: a 3D-multi-gradient-echo sequence with oxygen challenge (oxygen vs medical-air) to obtain 3D-maps of the T2* signal. E21 T2*-values were calculated for manually defined 3D-ROIs with custom-Matlab using the SQEXP-algorithm. In a separate cohort of rats, feto-placental vascular structural casts were quantified using micro-CT with Amira and customed Matlab analytic software for structural comparison. E21 fetal and placental weights and size mesures were reduced in Dex-treated rats ($p<0.05$). MRI scans revealed a higher ability to adaption to oxygen challenge test (whole-placental T2*) in Veh than Dex ($p<0.05$) when shifting from oxygen to medical-air. Importantly, the relative shift in whole-placental T2* differed significantly between Veh and Dex (3.1±0.4 vs 2.0±0.5 msec; $p<0.05$), indicating reduced blood oxygenation in Dex placentas, thus functional compromise. Furthermore, MRI structural measures aligned with a marked decline in feto-placental vascular complexity in Dex. The results of this proof-of-concept study demonstrate that T2*-based measurements of the placental blood oxygenation can provide non-invasive functional assessments of *in-vivo* placental health. Both structural and functional findings converge. Ongoing analyses are conducted to determine whether these changes are dynamic across gestation.

The Pathogenesis of Steroid-induced Osteonecrosis of Femoral Head is Mediated by Reactive Oxygen Species Mediate Via Enhancing Osteoclast Activity

Kai Chen[1], Yuhao Liu[1,2], Vincent Kuek[1], Nathan Pavlos[1], Wei He[2], and Jiake Xu[1]

[1]School of Biomedical Sciences, The University of Western Australia, Perth, WA, Australia, [2]Department of Joint Orthopaedic, the First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China

Introduction. Osteonecrosis of the femoral head (ONFH) is a progressive bone disorder which is characterized by subchondral microfractures at an early stage and femoral head collapse at a late stage. It is widely accepted that hyperactive osteoclasts, rather than the necrosis itself, causes the loss of bone structural integrity and hip joint dysfunction. However, the mechanisms underlying the activation of osteoclasts in the development of ONFH remain unclear. **Problem Statement.** Given the accumulating evidence showing reactive oxygen species (ROS) are positively related with osteoclast formation and function, we hypothesized that ROS may induce the hyperactivation of osteoclasts in the pathogenesis and progression of ONFH. **Procedures.** ROS and osteoclast markers were examined in both human osteonecrotic femoral heads and steroid-induced rat ONFH models using Western Blot (WB) assay and qPCR. Histomorphometric analyses and micro-CT were performed to observe the bone microstructures of the femoral head. **Results.** Histomorphometric analyses of decalcified human femoral head sections showed that necrotic bones were characterized by an absence of osteocytes within the lacunae and widely surrounded by osteoclasts. QPCR and WB analyses revealed that necrotic bone tissues expressed lower levels of antioxidant enzymes, but higher levels of osteoclast-specific markers compared with the control group. In the steroid-treated group, ROS fluorescence intensity of the femoral head was dramatically enhanced in comparison with the control group. WB analysis revealed that osteoclast-related proteins (RANKL and cathepsin K) were significantly up-regulated, which was accompanied by down-regulated expression of antioxidant enzymes, such as heme oxygenase 1 (HO-1), catalase, and SOD1.**Conclusions.** The steroid may induce the progression of ONFH by inhibiting the antioxidant enzymes, which may lead to a high level of ROS and subsequent enhancement of osteoclast activity.

Gene Mining the Spinal and Dento-skeletal Phenotypes of the Collaborative Cross

Samuel Bennett[1], Eason Guo[1], Jacob Kenny[1], Jennifer Tickner[1], Curtise Ng[2], Grant Morahan[3], Jiake Xu[1]

[1]School of Biomedical Sciences, University of Western Australia, [2]School of Molecular and Life Sciences, Curtin University, [3]Centre for Diabetes Research, University of Western Australia

The Collaborative Cross (CC) is a purpose-built genetics resource that was developed to enhance quantitative trait locus (QTL) and systems genetics analyses in mice. The Gene Mine (of the CC) housed at the Animal Resources Centre (Perth, WA), since 2004. Research into the osteoporosis, osteoarthritis, and scoliosis fields of the CC has been initiated. Our project is investigating the determinants of dento-skeletal phenotypes of the Gene Mine CC population. Our aims are 1) to identify novel molecular determinants of dento-skeletal homeostasis and disease in mice, and 2) to correlate these findings with concomitant disease phenotypes including osteoporosis, osteoarthritis, and scoliosis in CC mice. Our pioneering research will deliver the purpose of the CC: to advance the field of systems genetics research by advancing knowledge of the QTLs responsible for the osseous disease phenotypes representative of human populations. Conventional x-ray and micro-computed tomography for dento-skeletal, scoliosis, and kyphosis phenotypes screening and analysis. Mapping of QTLs will be performed to identify candidate genes. Gene expression and bioinformatics analyses will be used to verify and characterize candidate gene involvement. Conventional X-ray screening of 1137 CC mice across 84 strains from The Gene Mine has been completed. A range of phenotypes have been observed including scoliosis (5.2%), kyphosis (4.3%), kypho-scoliosis (1.05%), dento-skeletal (21.6%). Conventional x-ray screening results are consistent with the incidence of these pathologies in human populations, validating this approach, and indicating the need for further micro-CT analysis, identification and mapping of QTLs, and determination of candidate gene involvement for dento-skeletal, scoliosis and kyphosis phenotypes. The genomes of the Gene Mine CC mice are fixed, ensuring that the subsequent QTL mapping of disease phenotypes is powerful, accurate to high resolution, and representative of the genetic characteristics of human populations, and will advance our knowledge and potential to treat a range of diseases.

B5

Investigating the Impact of Acute Muscle Injury-Induced Inflammation on the Brain in the Elderly

Fiona Gearon[1], Dr. Connie Jackaman[2]

[1]Curtin University, [2]Curtin Health Innovation Research Institute

Introduction. A serious complication of acute injury including falls in elderly people is acute onset delirium and cognitive decline. 27% of elderly patients with acute musculoskeletal injury go on to develop delirium, a condition which increases their risk of developing dementia 3-5 times. **Problem Statement**. It is hypothesised that macrophages are a key driver of dysregulated systemic inflammation following acute injury, impacting on blood-brain barrier integrity and leading to neurological pathology in elderly subjects. **Procedures**. Young (2-3 months) and elderly (22-24 months) mice received notexin injections into both anterior tibialis muscles to produce acute injury. Ability to walk over 30cm was assessed daily over 4 days. Cohorts of both groups received macrophage depletion via intraperitoneal anti-F4/80 antibody injection daily over 4 days post-injury. Brain samples were collected from healthy, injured, and macrophage depleted mice after the 4 days and cryosections are being assessed via immunohistochemistry for IgG extravasation, blood-brain barrier integrity, astrocytes and microglia. Snap-frozen samples will also have bead array analysis performed for cytokine and chemokine levels for correlation with previously collected plasma levels and frailty scores. **Results**. Current data collected is limited to semi-quantitative analysis for IgG extravasation for one set of samples and appears to show raised inflammation in elderly mice even in healthy controls, average IgG being 250 compared to 164. This holds for injured mice, with average results of 191 in the young mouse and 243 in elderly. Macrophage depletion appears to reduce IgG extravasation in the elderly mouse, with a result of 198, however the young macrophage depleted mouse has a result of 208. **Conclusions**. It is impossible to draw definite conclusions while data collection is ongoing but current results are consistent with a state of increased inflammation being present following injury and macrophages impacting upon this state.

B6

Identifying Drivers of Invasion in a Murine Model of Malignant Pleural Mesothelioma

Lizeth Orozco[1,2], Catherine Rinaldi[1,2,5], Emma DeJong[3], Sally Lansley[6], Danika Hope[1,2], Thomas Casey[1,2], Gary Y Lee[6], Matthew Piggott[1], Alice Vrielink[1], Anthony Bosco[3], Joel Gummer[4], Richard Lake[1,2], Joost Lesterhuis[1,2,3]

[1]The University of Western Australia, [2]National Centre for Asbestos Related Diseases, [3]Telethon Kids Institute, [4]Murdoch University, [5]Centre for Microscopy Characterisation and Analysis, [6]Institute for Respiratory Health

Introduction. Malignant pleural mesothelioma is a cancer that originates in the pleura of the lungs and invades the surrounding tissues. Its low survival rate, 8-14 months post-diagnosis, is directly related to the aggressive local growth of the tumour cells. To this day, it is still unknown what drives mesothelioma invasion as well as how to overcome the progression of this disease. **Aims.** To use an invasive murine model to identify molecules that drive tumour invasion, and to validate them as targets for mesothelioma treatment in a murine model. **Procedures.** Syngeneic mesothelioma cell lines AB1 and AE17 were inoculated in BALB/c and C57BL/6 mice, respectively; either subcutaneously, or intrapleurally. Pleural and subcutaneous tumours derived from these cell lines were compared using RNA sequencing and metabolomics analyses. In vitro assays and molecular biology techniques were used to confirm the therapeutic inhibition of molecules of interest. **Progress and future approach.** Pleural tumours showed markedly increased growth and invasion compared to subcutaneous tumours. Combined RNA sequencing and metabolomics analyses of these tumours identified a metabolic program associated with invasive growth, with peroxisome proliferator-activated receptors (PPARs) as potential key regulators. In vivo studies targeting PPARs to inhibit mesothelioma invasion are currently ongoing.

B7

Airway Smooth Muscle Mechanosensation at Low and High Micro-environmental Stiffness

Tan YH[1], Choi YS[1], Sanderson RW[2], Li J[2], Chin IL[1], Wang KCW[1,3] and Noble PB[1]

[1]School of Human Sciences, The University of Western Australia, Crawley, [2]Harry Perkins Institute of Medical Research, [3]Telethon Kids Institute

Introduction. Increased airway stiffness is an established pathophysiological feature of airway disease that likely contributes directly or indirectly to airflow limitation and symptoms of breathing difficulties. Our research program is examining whether pathways of mechanosensation contributes to abnormal airway smooth muscle (ASM) function. **Aim**. To define reference values for airway stiffness and provide proof of concept that ASM cells respond accordingly to different levels of micro-environmental stiffness. **Methods**. Porcine trachea (n=6) was dissected to isolate the ASM layer. Stiffness (kPa) was measured by compressive-testing (Instron) utilizing a custom-designed platform developed by computer-assisted-design and 3D-printing. Measurements were performed at three levels of ASM stretch (0%, 5% and 10%, applied longitudinally). In separate experiments, 2D hydrogels were manufactured at three stiffness levels: 7, 17 and 35 kPa. Human ASM cells (hASMC) were cultured on these hydrogels for 4 days. Immunohistochemistry staining for nuclear and cytoplasmic expression of yes-associated protein (YAP) and alpha-smooth muscle actin (α-SMA) in cultured hASMCs was performed. Data were expressed as mean ± SEM. **Results**. Mean tracheal ASM stiffness at 0%, 5% and 10% stretch was 9.41 ± 2.68 kPa, 17.7 ± 5.51 kPa and 18.43 ± 6.17 kPa respectively (P=0.183). In culture, there was no significant increase in nuclear to cytoplasmic ratio of YAP expression with increasing gel stiffness (P=0.172). However, cytoplasmic expression of α-SMA increased with increasing gel stiffness (P=0.0001). Cell size was greater with increased gel stiffness: 1277 ± 140.9 μm^2 at 7 kPa, 2635 ± 610.7 μm^2 at 17 kPa and 5980 ± 880.9 μm^2 at 35 kPa (P<0.0001). **Conclusions**. The developed measurement platform successfully characterised *ex vivo* stiffness of tracheal ASM. *In vitro* culture of hASMCs provided preliminary evidence that ASM is mechanosensitive at stiffness levels present during physiological stretch of the ASM layer.

B8

Isolation and Characterisation of Staphylococcal Phages and Assessment of Their Therapeutic Potential

Maria Raymundo[1,2], Matthew Payne[1], Barbara Chang[2] and Lucy Furfaro[1]

[1]Division of Obstetrics and Gynaecology, The University of Western Australia

[2]Marshall Centre for Infectious Diseases Research and Training, The University of Western Australia

Methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* commonly colonise neonatal skin which can then lead to skin and soft tissue infection. Decolonisation agents such as antibiotics are often broad-spectrum and impact commensal bacteria causing dysbiosis. Bacteriophages (phages) present a promising targeted alternative. We aimed to isolate and characterise phages active against *Staphylococcus* spp. as potential decolonisation agents. In order to select a panel of host strains suitable for enrichment and screening of water samples for the presence of virulent phages, it is important to choose non-prophage-carrying strains. Clinical isolates of MSSA (n=44), MRSA (n=54) and *S. epidermidis* (n=11) were assessed for inducible prophages by mitomycin C induction and plaque assays. Of the 109 isolates, 77.3% of MSSA, 83.3% of MRSA and 100% of *S. epidermidis* were shown to contain one or more prophages inducible by this method. Those lacking an inducible prophage included 10 MSSA and 9 MRSA isolates and formed the screening panel, which was then used to enrich and screen wastewater collected across four sites (Subiaco, Beenyup, Gordon Road and Pinjarra). Thirteen phages with anti-*S. aureus* activity were isolated and purified. Enrichment with MRSA strain SSCC-56280 resulted in the isolation of a phage from each wastewater source and thus was the propagating host for 30.7% of the phages. 89% of the remaining phages (n=9) were isolated following enrichment with MSSA hosts. A total of 61.5% (n=8) phages were isolated in more than one host, primarily in MSSA isolates. Plaque morphology varied depending on the host and included clear, turbid and haloed plaques. In summary, we have isolated 13 phages with activity against both MSSA and MRSA clinical isolates that may present promising alternatives to antibiotic treatment. Further genomic and phenotypic analysis will be important for determination of their therapeutic potential in neonatal skin decolonisation.

Cell-cycle Checkpoint Inhibition in Combination with Targeted Radiotherapy Enhances Survival in Models of High-risk Medulloblastoma

Hilary Hii[1], Brooke Strowger[1], Meegan Howlett[1], Jacqueline Whitehouse[1], Nicholas G Gottardo[1,2] and Raelene Endersby[1].

[1]Telethon Kids Cancer Centre, Telethon Kids Institute, and the University of Western Australia; [2]Department of Paediatric and Adolescent Oncology/Haematology, Perth Children's Hospital.

Introduction. Medulloblastoma is the most common malignant brain tumour of childhood. Four molecularly distinct subgroups exist, and each respond differently to current therapy. Treatment is multimodal involving surgical resection and targeted radiotherapy, followed by chemotherapy. Despite this aggressive treatment regime, survival rates for high-risk medulloblastoma remains dismal and better treatments are urgently needed. **Problem Statement**. Prexasertib, an inhibitor of the cell cycle checkpoint kinases 1 and 2 (Chk1/2), is a regulator of the DNA-damage response pathway. Our team has demonstrated that this compound enhances the effects of chemotherapy, resulting in increased tumour cell death and extended survival in mouse models of medulloblastoma. Our goal in this study was to determine if administering prexasertib with targeted radiotherapy extends survival in mouse models of high-risk medulloblastoma. **Procedures**. The XRAD image-guided small animal radiotherapy (SmART) system combines CT imaging with precision irradiation using x-rays. The acute effects of targeted radiotherapy were analysed by delivering one dose of 2 Gy irradiation in combination with 20mg/kg of prexasertib. Markers of DNA damage and apoptosis were used to evaluate tumour targeting. In order to model multifractionated radiotherapy, we have trialed 9, 18 and 36 Gy multifractionated radiotherapy and compared the effects of multifractionated radiotherapy in combination with prexasertib. **Results**. Two models of high-risk medulloblastoma (MycP53^{DD} and BT084) showed significant increases in apoptosis and DNA damage in the prexasertib plus radiation treatment group when compared to radiation alone ($p<0.05$). Moreover, this combination significantly extended survival when compared to all other treatment groups ($p<0.0001$) in an additional model of group 3 medulloblastoma (D425). **Conclusions**. Prexasertib enhances the effects of radiation in mouse models of high-risk medulloblastoma, highlighting the potential for the combination of prexasertib and targeted radiotherapy to increase treatment efficacy in the most aggressive forms of medulloblastoma in the clinic.

CHK Kinase Inhibition Amplifies the Effects of Chemotherapy in Pineoblastoma

Jessica Buck[1], Clara Andradas[1], Hetal Dholaria[1], Hilary Hii[1], Brooke Strowger[1], Mani Kuchibhotla[1], Nick Gottardo[1], and Raelene Endersby[1].

[1]Brain Tumour Research Program, Telethon Kids Cancer Centre, Telethon Kids Institute

Introduction. Pineoblastoma is a rare paediatric brain tumour with a survival rate of only 50%. In a high-throughput drug screen, kinase inhibitors targeting multiple cell cycle regulators were identified as effective in this disease. Inhibitors of the cell cycle checkpoint kinases 1 and 2 (CHK1/2) have shown promise in sensitising several cancers to the effects of chemotherapy and radiotherapy, thus improving survival, but have not previously been tested in pineoblastoma. **Problem Statement**. There is an urgent need to find improved treatments for pineoblastoma. The aim of this study was to test whether the CHK1/2 inhibitor prexasertib could inhibit cell cycle checkpoints in an *in vivo* mouse model of pineoblastoma following exposure to DNA-damaging chemotherapy. **Procedures**. Mice were implanted with TK-PB452A (pineoblastoma) or TK-PB453 (recurrent pineoblastoma) cells. Following tumour growth, a single dose of the CHK1/2 inhibitor prexasertib was administered, alone or in combination with chemotherapy (gemcitabine or cyclophosphamide), and brains were harvested after 24 hours. Pathway inhibition, along with markers of response to chemotherapy, such as DNA damage and apoptosis, were detected using immunohistochemistry and quantified. **Results**. Prexasertib mediated inhibition of CHK1/2 kinase activity was confirmed in both orthotopic xenograft models by detection of increased phospho-CHK1-S345 ($p<0.0001$) and decreased phospho-CHK1-S296 ($p<0.01$) in the TK-PB452A model. DNA damage was also increased in both models following prexasertib administration as measured by gamma-H2AX ($p<0.01$). Apoptosis, as measured by cleaved caspase-3, was also increased ($p<0.0001$) in the TK-PB452A model. **Conclusions**. Prexasertib effectively inhibited the CHK1/2 pathway in a mouse model of pineoblastoma, and potentiates the effects of chemotherapy by enhancing DNA damage and cell death. These data suggest that chemotherapy can be enhanced using prexasertib to potentially increase treatment efficacy and reduce disease recurrence. Ongoing work is testing whether prexasertib can increase survival in combination with chemotherapy and radiotherapy.

C3

Colostrum Drives the Development of Successful Anti-Helminth Immune Defenses

Akila Rekima[1], Charlotte Isnard[2], Danielle J. Smyth[3], Rick M. Maizel[3], Valérie Verhasselt[1,1]

School of Molecular Sciences, University of Western Australia, [2]Neonatology Hopital de l'Archet II, France,[3] Institute of Infection and Immunology Research & Centre for Immunity, Infection & Evolution, School of Biological Sciences, University of Edinburgh, United Kingdom

Colostrum is produced during the first 2 -3 days by the mammary gland and profoundly differs both qualitatively and quantitatively from mature breast milk. It is enriched in antibodies, growth factors, vitamin, and oligosaccharides. Strikingly, among breastfeeding mothers, there is a worldwide lack of colostrum administration in more than 50% of cases. A recent large scale study in developing countries showed a major negative impact of delayed initiation of breastfeeding on neonatal mortality and morbidity up to 6 months of age. To elucidate by which mechanisms colostrum mediates health benefits, we addressed its impact on gut mucosal immune ontogeny in a mouse experimental setting. Mice were breastfed by mothers providing from birth either physiological feeding, i.e. colostrum from followed by mature milk, or only mature milk. At the time of weaning, we found an increased gut permeability, a decreased representation of goblet cells, innate lymphoid group 2 and Th2 cells in the small intestine *lamina propria* of mice fed with mature milk from birth compared to mice fed with colostrum. This improper development of gut mucosal immunity resulted in an increased susceptibility to infection by the intestinal helminth parasite, Heligmosomoides polygyrus. In conclusion, our data highlight that colostrum may be specifically designed to satisfy the needs of the developing newborn and be critical for type 2 immunity in early post-natal life.

C4

Colostrum is Key for Optimal Growth

Lieke van den Elsen[1], Akila Rekima[1], Charlotte Isnard[2], Valerie Verhasselt[1]

[1]School of Molecular Sciences, The University of Western Australia, Perth, WA [2]University of Nice, Nice, France

Nearly half of all deaths in children under 5 years is attributable to undernutrition. Undernutrition can also be associated with lifelong consequences such as poor cognition. Colostrum, the first fluid produced by the mammary gland, is very rich in protein and growth factors. It also has the potential to shape gut microbiota establishment by specific oligosaccharides and antimicrobial factors. Despite its rich composition, colostrum-feeding practices are suboptimal. We propose that the lack of colostrum feeding contributes to growth retardation in association with gut microbiota dysbiosis and leaky gut. To address this we compared the growth of mice fed physiologically (control group) with mice fed by a dam that delivered 8 days earlier and thus provided mature milk only. Mature milk mice demonstrated reduced body weight gain, crown-to-rump length and abdominal width compared to control mice from as early as 3 days old. The weight difference peaked around day 5, with 35% deviation from the control group. *In vivo* quantification of subcutaneous and visceral white adipose tissue (WAT) using MicroCT demonstrated that the visceral WAT was the fat depot most affected by the lack of colostrum feeding at day 15. This was accompanied by a marked difference in gut microbial diversity and composition between mice fed with or without colostrum. The mature milk group also showed an increase in gut permeability. Our data suggest that colostrum is critical for physiological growth in early post-natal life. This may be associated with gut microbiota dysbiosis which can disrupt the gut barrier and lead to an inflammatory phenotype and poor growth. Further experiments will determine whether immune dysfunction in WAT due to gut dysbiosis is responsible for the growth defect in mice deprived of colostrum. Ultimately, this work should provide the basis to promote colostrum feeding and/or design colostrum-inspired supplements to support healthy growth.

Combining ATR Inhibition with Chemotherapy and Radiation Therapy Enhances Cytotoxicity in Group 3 Medulloblastoma

Brooke Strowger[1], Meegan Howlett[1], Jacqueline Whitehouse[1], Mani Kuchibotla[1], Hilary Hii[1], Courtney George[1], Nicholas Gottardo[1,2] and Raelene Endersby[1].

[1]Telethon Kids Cancer Centre, Telethon Kids Institute, [2]Department of Paediatric and Adolescent Oncology/ Haematology, Perth Children's Hospital.

Introduction. Medulloblastoma is the most common malignant brain tumour of childhood. Surgical resection and craniospinal irradiation followed by chemotherapy are the mainstay of treatment. Despite treatment intensification, survival has plateaued for the past two decades at around 70% and patients that relapse are essentially incurable. **Problem Statement**. To identify novel drugs that can enhance frontline therapies and increase cure rates as a result. **Procedures**. An unbiased high-throughput drug screen identified inhibitors of the DNA-damage response pathway as promising candidates, including kinase inhibitors targeting ATR. ATR is a key mediator of the pathway and its activation allows tumour cells to repair otherwise fatal damage caused by the therapy. We tested the ability of an inhibitor of ATR (iATR) to kill group 3 medulloblastoma tumour cells using *in vitro* drug interaction assays. *In vivo* testing was conducted using sophisticated, orthotopic mouse models of medulloblastoma. Clinical radiation protocols were also mimicked in our mouse models using the state-of-the-art XRAD SmART system. **Results**. iATR enhanced *in vitro* cytotoxicity of conventional chemotherapeutics cisplatin and cyclophosphamide as well as gemcitabine, which is currently in clinical trial. When given in combination with conventional chemotherapy, iATR significantly extended survival in several different medulloblastoma mouse models. We also found that iATR can enhance radiation induced tumour cell death. **Conclusions**. We highlight the exciting new potential of iATR as an adjuvant frontline therapy. Future studies will determine if iATR can facilitate a reduction in the dose of harmful radiation without compromising survival.

Could Exon Skipping Become a Treatment for Recessive Ullrich Congenital Muscular Dystrophy?

Craig, McIntosh[1], Sue Fletcher[1,2], Steve D Wilton,[1,2] May Thandar Aung-Htut[1,2]

[1]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, [2]Perron Institute for Neurological and Translational Sciences

COL6 related congenital muscular dystrophy (CMD) is caused by mutations in either COL6A1, COL6A2 or COL6A3 genes that disrupt the normal assembly of the COL6A1, COL6A2, and COL6A3 chains into heterotrimer fibrils essential for integration into the extracellular matrix. Autosomal recessive Ullrich CMD patients carrying mutations that lead to premature termination of translation present with severe phenotypes due to extremely low levels or lack of functional COL6. This is a proof-of-concept study to ascertain if the exon skipping therapy developed for the treatment of Duchenne muscular dystrophy could be applied to bypass mutated/disease-causing exons and restore some functional COL6 production in Ullrich CMD patients. We explored whether: (i) small exons in COL6 transcripts can be efficiently and specifically excised; (ii) the equivalent exons in COL6A1, COL6A2, and COL6A3 gene transcripts can be simultaneously excised; and (iii) these modified COL6A isoforms can assemble into functional trimer fibrils. We analyzed COL6 protein assembly and designed antisense oligonucleotides to induce skipping of the corresponding exons of COL6A1, COL6A2, and COL6A3 transcripts, which encode part of the triple helix structure of COL6. The initial screen was performed using oligomers synthesized as 2'-O-methyl modified bases on a phosphorothioate backbone. The best performing sequences were then synthesized as the more efficient and clinically relevant, phosphorodiamidate morpholinos oligomers. Efficient exon 23 skipping of the COL6A2 transcript in healthy fibroblasts disrupted COL6 assembly as expected, indicating that the exon skipping we observed at the RNA level was translated into protein. We are currently refining antisense oligomers which induce exon skipping of corresponding exons in COL6A1 and COL6A3 transcripts to establish if multi-exon skipping can restore some functional assembly of COL6.

This work is supported by Murdoch University, Western Australia and Million Dollar Bike Ride Pilot award (2019), Orphan Disease Centre, University of Pennsylvania, Philadelphia, United States.

C7

Detection of Bile Acids in the Lungs of Paediatric Cystic Fibrosis Patients is Associated with Inflammation and Lung Microbiota Remodelling

Jose A. Caparrós-Martín[1], Stephanie Flynn[2], F. Jerry Reen[2], David F. Woods[2], Patricia Agudelo-Romero[3], Stephen M. Stick[3] and Fergal O'Gara[1,2,3] On behalf of AREST CF

[1]Human Microbiome Programme, School of Pharmacy and Biomedical Sciences and Curtin Health Innovation Research Institute, Curtin University. [2]BIOMERIT Research Centre, School of Microbiology, University College Cork, Cork, Ireland. [3]The Telethon Kids Institute, Perth WA.

Cystic fibrosis (CF) is a genetic disorder in which sustained inflammation and recurrent infections play dominant roles in disease progression. Recent research has shown that inflammation, infection and lung structural damage start early in life, even before clinical symptoms become evident. There is a growing need for better therapeutic intervention to prevent airway infection and inflammation to reduce morbidity and extend life expectancy. The study of young patients provides a unique opportunity to dissect the molecular mechanisms involved in the early stages of CF lung disease. Having access to a unique paediatric CF patient cohort, we evaluated whether the presence of bile acids in the lungs of clinically stable children diagnosed with CF is associated with inflammation, microbial infection and clinical outcomes. Using an unsupervised classification approach based on the detection of bile acid profiles, we demonstrated associations linking bile acids to the disruption of immune and microbial homeostasis. Upon closer evaluation of the lung bacterial communities of this CF cohort, we observed that the presence of bile is associated with a remodelling of the biological relationships between microorganisms of the lung ecosystem. These changes were characterized by a reconfiguration of the ecological interactions between species, with pathogens occupying potentially relevant positions within the community. This observed behaviour suggests that bile acids may be a triggering factor influencing the establishment of chronic infections in the lungs. Supported by a statistical model, we also validated bile acid detection as a biomarker for predicting the progression of CF lung disease. Overall, our work suggests that bile acids could play a key role in modulating early events in the aetiology of CF lung disease.

C8

Houston, the T-DNA has landed! *Agrobacterium tumefaciens* mediated transformation (ATMT) of *Ascochyta lentis* to monitor ascochyta blight progression on resistant and susceptible lentil genotypes.

Johannes W. Debler, Bernadette M. Henares, Lina Farfan-Caceres, Christina R. Grime, Robert C. Lee

Curtin University, Centre for Crop and Disease Management

Introduction. The Australian lentil industry produces an average of 292,000 tonnes annually. Ascochyta blight, caused by the necrotrophic fungal pathogen *Ascochyta lentis*, leads to annual yield and quality losses of A$0.9 M and control costs add a further A$15.3 M in penalties to the Australian lentil industry. **Problem Statement**. In order to investigate differences between resistant and susceptible lentil genotypes we needed a fluorescently labelled *A. lentis* strain to observe the progression of ascochyta blight at various stages of host colonisation. **Procedures**. We established a protocol to transform *A. lentis* with green fluorescent protein (GFP) using ATMT and characterised the transgene insertion via Illumina short read sequencing. We followed the infection process via confocal laser scanning microscopy and identified key differences between resistant and susceptible genotypes. **Results**. The early stages of infection with no observable necrotic lesions looked the same for both genotypes (0-5 days post infection, dpi). Spores could be observed germinating and forming hyphae on the leaf surface. Penetration attempts were evident by the formation of penetration structures such as germ tubes and appressoria and white fluorescence around the penetration sites. At 7 dpi, when the first macroscopic lesions were visible, fungal proliferation within the leaf tissue and changes to hyphal morphology could be observed for the susceptible genotype while hyphae continued to grow and attempted penetration of the resistant one. At 9 dpi severe infection was clearly visible on the susceptible genotype and sporulation had begun while the resistant genotype was free from necrotic lesions and only few fluorescent hyphae could be observed. **Conclusions**. We successfully established a protocol to transform *A. lentis* which allowed us to study disease progression via fluorescent markers and opens the door for further study of fungal pathogenicity genes in the future.

C9

Improved Therapy For Paediatric Pineoblastoma By The Disruption of Cell Cycle Control

Clara Andradas[1]*, Hetal Dholaria[1, 2]*, Mani Kuchibhotla[1], Suresh Gande[1], Jacqueline Whitehouse[1], Brooke Strowger[1], Jessica Burk, Tobias Schoep[1], Courtney George[1], Hilary Hii[1], Nick Gottardo[1, 2], Raelene Endersby[1]. * equal

[1] Telethon Kids Institute, Nedlands, WA, Australia; [2] Perth Children's Hospital, Nedlands, WA, Australia

Introduction: Pineoblastoma is an invasive embryonal brain tumour arising in the pineal gland of children. Since they are rare, limited clinical trials specific for these patients have been performed. As such, pineoblastoma patients receive the same treatment as medulloblastoma, another more common embryonal brain cancer, however the biology and behaviour of these tumours is known to be diverse. As a consequence, the outcomes of children with pineoblastoma are significantly worse, with survival rates of approximately 65%. Improved therapies for pineoblastoma are urgently needed. A lack of preclinical models has hindered development of more effective therapies for pineoblastoma. **Objective**: our aim is to identify new therapeutic strategies that could improve the outcomes of pineoblastoma patients. **Procedures**: in this context, we performed different *in vitro* and *in vivo* approaches with two cell lines from an 8-month-old girl with extensive pineoblastoma. These cells robustly form pineoblastoma upon intracranial implantation in mice. **Results**: we performed a high-throughput drug screen that identified DNA damage response inhibitors, especially cell cycle checkpoint kinase 1 and 2 (CHK1/2) inhibitors as promising candidates for pineoblastoma treatment. Here, we demonstrate a synergistic interaction of these inhibitors with conventional pineoblastoma chemotherapies both *in vitro* and *in vivo*. CHK1/2 inhibitors were found to impair cell cycle arrest after chemotherapy-induced DNA damage, increasing pineoblastoma cell death *in vitro*. Importantly, the combination of CHK1/2 inhibitors with DNA-damaging chemotherapy significantly extended the survival of mice with pineoblastoma. **Conclusion**: our data show that this could be an effective new therapeutic combination for paediatric pineoblastoma and suggests patients may benefit from inclusion in the upcoming SJ-ELiOT clinical trial combining the CHK1/2 inhibitor prexasetib with conventional chemotherapies.

C10

Improvement of a Real-time PCR-based Assay for Quick Detection and Quantification of Monodon Baculovirus of Tiger Shrimp (*Penaeus nonodon*) in Southern Vietnam

Truc T Huynh[1], Sang D Huynh[1,*], Huong XT Bao[1], Phi H Pham[1], Thu H Bui[1]

[1] Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam,
[*]Currently a PhD student at the ARC Center of Excellence in Plant Energy Biology, School of Molecular Sciences, The University of Western Australia, Australia

Shrimp farming contributes significantly to the agriculture economy in Vietnam. However, intensive cultivation of tiger shrimp (*Penaeus monodon*) allows fast transmission of viral diseases between farms. Economic loss for farmers is unavoidable when diseases occur. monodon baculovirus (MBV) is one of the most dangerous viruses of shrimp that can cause death rate of up to 70%. Development of methods for quick MBV detection at early stages after infection of broodstock and shrimp larvae is crucial to prevent the disease to occur and spread. In this research, samples showing symptoms of MBV DNA was amplified with sequence specific primers and cloned into the pGEM-T Easy vector. Positive clones were selected on LB plates containing ampicillin/X-gal/IPTG and after sequencing were used as a template to produce a standard curve. To optimise primer concentration for SYBR green-based Real-time PCR an experiment was conducted with different primer concentrations. The detection limit of the Real-time PCR assay was tested by series of standard from 10^7 to 10^0 copies. The standard curve of a Real-time PCR was also generated. The results of our research show that the Real-time PCR assay based on SYBR green provides high sensitivity as it could detect MBV at a level of 100 copies. The standard curve also shows high reliability for MBV quantitation as its estimated linear correlation coefficient (r^2) was 0.994364. This assay could be utilized to quickly and precisely screen for the presence of MBV in broodstock and shrimp larvae, therefore it can potentially contribute to virus-free tiger shrimp breeding in Vietnam.
Key words: Monodon baculovirus (MBV), Real-time PCR, SYBR green, shrimp.

C11

Phosphorothioate Oligomer Sequestration of Paraspeckle Proteins and Formation of Toxic Nuclear Structures

Loren L Flynn[1,2], Ruohan Li[3], May T Aung-Htut[1,2], Ianthe L Pitout[1,2], Jack Cooper[3], Alysia Hubbard[3], Lisa Griffiths[4], Charlie Bond[3], Steve D Wilton[1,2], Archa H Fox[3] and Sue Fletcher[1,2]

[1]Centre for Comparative Genomics, Murdoch University, [2]Perron Institute for Neurological and Translational Science, [3]University of Western Australia, [4]Department of Health, Western Australia

Phosphorothioate oligonucleotides are commonly used antisense compounds for inducing RNase H degradation and modifying pre-mRNA splicing in amenable diseases. However, phosphorothioate oligomers have been shown to interact non-specifically with a number of proteins leading to concerns regarding their off-target effects on cellular biology. Here, we report that 2´ O-methyl phosphorothioate oligomers sequester nuclear paraspeckle proteins, forming large fibril-like structures that dominate the nucleoplasm resulting in nuclear stress and caspase activation. These findings were irrespective of oligomer sequence or nucleotide composition, as over 100 2´ O-methyl phosphorothioate oligomers transfected into fibroblasts induced nuclear structures and paraspeckle protein disruption. Transmission electron microscopy of phosphorothioate oligomer transfected fibroblasts, revealed these highly organized fibril/amyloid-like structures were up to ~2000 nm in length and ~250 nm in diameter. These structures were highly stable following cell death and appeared to physically disrupt and constrain nuclear architecture. RNA-seq analysis confirmed global disturbances in cellular biology as a result of these nuclear structures, including the disruption of RNA processing, cell signaling and autophagy, adding to the growing concern for the clinical application of phosphorothioate oligomers.

C12

Statins are Associated with an Increased Risk of Skin Infections: A Sequence Symmetry Analysis of Prescription Claims.

Humphrey Ko[1,2], Ricky R Lareu[1,2], Brett Dix[1], Jeffery Hughes[1] and Richard Parsons[3]

[1]School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University (CU). [2]Curtin Health Innovation Research Institute (CHIRI) Biosciences Research Precinct, CU; [3]School of Occupational Therapy, Social Work and Speech Pathology, CU.

Introduction. Statins consistently top the list of most prescribed drugs and occupied the top two spots for Australia in 2017-2018. Used for primary and secondary prevention of cardiovascular disease, they have been linked to an increase in new-onset diabetes, and skin and soft tissue infections (SSTIs). However, the latter is still controversial. We used a relatively new approach with prescription data to analysis the relationship between statin administration, new-onset diabetes and SSTIs. In addition, we tested the hypothesis that statins increase the risk of SSTIs in diabetic patients. **Methods.** Sequence symmetry analysis (SSA) was performed on prescription claims (2001 to 2011) from the Australian Department of Veterans' Affairs to determine the interrelationships between (i) statins and SSTIs, (ii) statins and diabetes, and (iii) diabetes and SSTIs. Prescriptions for statins, antidiabetic medication, and anti-staphylococcal antibiotics were evaluated using non-identifiable client numbers, prescription dates filled, residential electorates, and pharmaceutical codes. Adjusted sequence ratio (ASR) and confidence interval (CI) were calculated at intervals of 91, 182, and 365 days for SSA studies. **Results**. Statins were associated with (i) a significant SSTI risk (ASR = 1.40 to 1.41; CI >1), (ii) a significant diabetes risk (ASR = 1.19 to 1.09, CI >1), and (iii) diabetic patients having an increased risk of SSTIs (ASR = 1.2 and 1.24; at 182 and 365 days; CI >1). Diabetic and non-diabetic statin users had significantly increased risks of SSTIs, while the influence from socio-economic status was not significant for each of the three relationships. **Conclusions**. Statins appear to be associated with an increased risk of SSTIs via direct and indirect mechanisms, likely independent of diabetes or socio-economic status. Clinicians should be aware of the association between statins and SSTIs and consider monitoring blood glucose levels in statin users, where appropriate.

The Pentatricopeptide Repeat Protein Family and Fertility Restoration in Wheat

Joanna Melonek[1], Hediyeh Tahghighi[1], Ruonan Zhou[2], Nils Stein[2, 3] and Ian Small[1]

[1]ARC Centre of Excellence in Plant Energy Biology, School of Molecular Sciences, The University of Western Australia [2] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Stadt Seeland, Germany, [3]School of Agriculture and Environment, The University of Western Australia

Introduction: Hybrid production in cereals requires a way to control self-pollination of plants. A system that has been successfully used for breeding hybrids in some plant species is based on cytoplasmic male sterility (CMS). CMS is induced by mitochondrially-encoded genes whose expression results in plant sterility. Nuclear-encoded *Restorer of Fertility* (*RF*) genes restore plant fertility by blocking expression of CMS-transcripts. The majority of to date identified *RF* genes form a distinct clade in the family of the pentatricopeptide repeat proteins (PPRs), sequence-specific RNA-binding factors located to organelles and involved in RNA maturation processes. The PPR family is one of the largest gene families in flowering plants and counts typically 550-700 PPR genes. **Problem Statement:** The lack of strong restorer genes hinders the development of CMS-based hybrid breeding systems in wheat. **Procedures and Results**: In the wheat reference genome we found 1686 PPRs, of which 207 were identified as *restorer of fertility-like* (*RFL*), far more than in any other plant genome analysed to date. The large number of PPR and RFL genes is primarily due to polyploidy and it's actually lower than expected from simply adding genes present in the progenitor diploid genomes. This implies PPR gene inactivation and loss during polyploidization. In addition, we show that locations of some of the previously mapped restorer genes overlap with the genomic locations of RFL clusters identified in our study. **Conclusions:** This is the first comprehensive analysis of the PPR and RFL family in wheat. The sequence knowledge gained from this project has the potential to accelerate hybrid wheat breeding programs by facilitating the identification of active restorer genes in potential restorer lines. Hybrid wheat varieties are expected to have higher and more consistent yields by better adaptation to increasingly unpredictable weather conditions in the era of global climate change.

Exogenous Antioxidant Treatments in the Cryopreservation of Recalcitrant Rainforest Species

Lei Hou[1], Bryn Funnekotter[1,2], Eric Bunn[2], Ricardo L.Mancera[1]

[1]School of Pharmacy and Biomedical Sciences, Curtin University, [2] Kings Park Science, Botanic Gardens and Park Authority

Recalcitrant seeded species cannot be preserved through conventional seed banking due to their low tolerance to dehydration and low temperatures. Cryopreservation, the storage of tissues in liquid nitrogen (LN), is instead considered the most reliable and cost-effective alternative. As part of the cryopreservation process, plant tissues must undergo desiccation and cryoprotective agents (CPAs) treatments to mitigate lethal ice formation. These treatments are stressful and often induce the formation of harmful reactive oxygen species. The addition of exogenous antioxidants could potentially be beneficial to survival if endogenous antioxidant production itself is inadequate to mitigating oxidative stress. Ascorbic acid (AsA) and glutathione (GSH) are common non-enzymatic and water-soluble antioxidants in plant antioxidant defence. Their involvement in the AsA-GSH cycle regulates hydrogen peroxide levels to prevent the occurrence of the extremely detrimental development of lipid peroxidation. *Syzygium paniculatum and Syzygium australe*, as recalcitrant seeded model rainforest species, were used to study the effect of AsA and GSH on pre- and post-cryogenic survival. Shoot tips and embryonic axes were exposed to either 0.3 µM GSH or 0.5 µM AsA prior to cryopreservation using the vitrification-based 'cryo-mesh' method. Exposure to CPAs without antioxidants resulted in roughly 75% survival while GSH and AsA exposure slightly improved shoot tip survival by 8% and 6% respectively. Embryonic axes treated by either AsA or GSH resulted in 100% survival and regeneration following CPAs exposure as compared to 90% survival for axes without antioxidants treatments. Nonetheless, neither antioxidant treatments resulted in tissue survival following the exposure to LN. Further optimisation of the cryopreservation protocol is therefore needed. The improved tolerance to CPAs following antioxidant treatments indicates that a longer exposure to the CPAs or increased concentrations of CPAs to limit ice formation would be beneficial, leading to a greater chance for achieving post-cryogenic success in these *Syzygium* species.

Targeted Mutagenesis in Tobacco Using CRISPR/Cas9

Anindita Chakraborty[1], Hua Li[1], Sadia Iqbal[1], Michael G K Jones[1], Stephen J Wylie[1]

[1] Western Australia State Agricultural Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, Perth, Australia

The CRISPR/Cas9 system is an RNA-guided genome editing tool consisting of a Cas9 nuclease and single guide RNA (sgRNA). This system evolved in bacteria to kill bacteriophages. It was recently modified and successfully applied to eukaryotic species for modification of specific genomic DNA sequences. The CRISPR/Cas9 system can be used to delete gene activity, or to correct faulty sequences and restore gene function. The present study was conducted on the model plant *Nicotiana tabacum* L. (tobacco). A transfer-DNA (T-DNA) cassette containing *Cas9* was introduced into tobacco. The *Cas9* gene was integrated into the tobacco genome with *bar*, a physiological marker gene that confers resistance to the herbicide glufosinate ammonium. An aim of the experiment was to test efficiencies of different methods to introduce sgRNAs to the tobacco plants. The *bar* gene was used as a target; effective targeting of *bar* will abolish tolerance to glufosinate ammonium. Five sgRNA molecules were designed to knock out *bar*, and various methodologies will be tested in which transient-expression of sgRNAs in the transgenic tobacco plants will generate edited plant lines. This research will provide methodologies in which efficiently express exogenous sgRNAs to plants enable gene editing.

Shifting Soil Fungal Communities in Response to Fire and Weed Management in Urban Banksia Woodlands

Aaron Brace[1], Joe Fontaine[2], Katinka Ruthrof[2], Ray Froend[1], Ben Miller[3], Anna Hopkins[1]

[1]Edith Cowan University, [2]Murdoch University, [3]Department of Biodiversity, Conservation and Attractions.

Issues arising from habitat fragmentation such as extirpation and loss of biodiversity are exacerbated by a warming and drying climate, land use changes and non-native species invasion. To maintain biodiversity various management methods are employed, methods such as prescribing fires or herbicide application. Many of these strategies are macroorganism focused, with less attention paid to microorganisms. Soil fungi play instrumental roles in ecosystem function, yet in many ecosystems not much is known about how soil fungi interact with plants to influence their survival and persistence. Ecosystems such as the Swan Coastal Plain's endangered Banksia woodland, where active fire and weed management are undertaken is one such ecosystem where there is a gap in the knowledge. This project will examine soil fungal communities through different fire intervals, post fire succession, weed and fire management. Soils will be collected from Banksia woodland sites across the Swan Coastal Plain under differing fire and weed management treatments. A glasshouse trail will also be run to examine the impact of different herbicide applications on soil fungi. For all studies, fungal communities will be examined using high throughput sequencing, grouped into operational taxonomic units (OTUs) and assigned putative function where possible. Comparisons between sites and treatments will be made and functional changes in fungal communities can be observed. Parallels can be drawn with changes occurring in plant communities, for an inference of co-occurrence/interactions between plant and fungal communities. This work occupies a novel space as no study has investigated the combined effects of fire management and herbicide application on the soil fungi community in Banksia woodland. This project will fit well into the wider research on the Banksia woodland, providing knowledge in an area that has otherwise been poorly studied. This knowledge can then be used to better inform management decisions and aid in conservation measures.

MicroRNA-7 is a Tumour Suppressor That Overcomes Cisplatin Resistance in Head and Neck Cancer

Rikki AM Brown[1,2], Felicity C Kalinowski[1], Kirsty L Richardson[1], Dianne J Beveridge[1], Patrick A Candy[1], Lisa M Stuart[1], Peter J Leedman[1,2]

[1]Harry Perkins Institution of Medical Research, UWA Centre for Medical Research, [2]School of Medicine, University of Western Australia

Introduction. Head and neck cancer is the seventh most common cancer in Australia, with over 5000 new cases diagnosed and roughly 1200 deaths per year (AIHW, Cancer in Australia 2019). Cisplatin, a chemotherapeutic, remains the standard of care for treating advanced HNC, but many patients either do not respond or develop resistance and for them the prognosis is poor. **Problem Statement**. New approaches to improve the efficacy of cisplatin or provide new alternative treatments would improve outcomes for HNC patients. **Methods**. We used a cell line model of cisplatin resistant HNC to identify microRNAs that overcome cisplatin resistance. We studied the mechanisms of action using proteomics and cell-based functional assays to assess survival, cell cycle and senescence. **Results**. We identified that microRNA-7 (miR-7) acts as a tumour suppressor and synergises with cisplatin to reduce cell viability. We found that overexpression of miR-7 in HNC cells halts cell cycle progression and induces senescence, as opposed to triggering apoptosis. We focused on a target of miR-7, RAF1, which was upregulated in cisplatin resistant cells and show that either pharmacological or siRNA targeted inhibition of RAF1 similarly overcomes cisplatin resistance. **Conclusions**. Therapeutic delivery of miR-7 or RAF1 targeted therapy could be used in the current regimen for the treatment of HNC and improve the prognosis for these patients.

Investigating the role of Sec61α and Sec63 through cancer associated mutations

Aleshanee Paxman[1,2], Dr. Carl Mousley[1,2], Dr Rob Steuart[2], Christopher Witham Author[2]

[1]School of Pharmacy and Biomedical Sciences, [2]Curtin Health Innovation Research Institute, Curtin University

In Eukaryotes, the endoplasmic reticulum (ER) is the entry point to the secretory pathway. Proteins enter through the translocon, a protein conducting channel in the ER membrane. The translocon is formed by the Sec61 complex, whereby Sec61α is the largest protein component within this, and is assisted in its function by Sec63. Aspects of translocation remain elusive, especially the essential role of the Sec63. During translocation there is the opportunity for small molecules, including Ca2+ to leak out of the ER. In higher eukaryotes, Ca2+ is stored in the ER and can be released to act as a cellular signal. Cancer mutations can alter ER Ca2+ homeostasis, manipulating cellular signalling to promote malignancy. The ability for the translocon to regulate the flux of molecules into and out of the ER represented an under explored process that could have profound effects on disease progression. The cancer database was mined, and mutations have been found within conserved residues of Sec61α and Sec63, and plasmid borne copies of these mutations were generated using site directed mutagenesis. *Saccharomyces cerevisiae* was utilised in this study as translocon components and processes are highly conserved through all eukaryotes. These mutations were then introduced to a previously charactered strain with defects in translocon gating. This strain displays a temperature sensitive (TS) growth phenotype due to its defects. Initial analysis has found several of these mutations to have dominant or recessive suppression of the TS phenotype. Additionally, other mutations were found to exacerbate these deleterious effects, indicating that progression of cancers can arise from radically diverse metabolic states. The stress response in these mutants will be assessed and specific gating assays will be utilised to further characterise the abilities of the mutants to affect gating. Further work will continue to characterise cancer associated mutations not yet assessed.

E3

Melanoma cell adhesion molecule (MCAM) and its role in melanoma metastasis

Jake Harfield, Danielle Dye, Deirdre Coombe

Curtin Health and Innovative Research Institute, School of Biomedical Sciences, Faculty of Health, Curtin University

Melanoma cell adhesion molecule (MCAM) is a key player in melanoma metastasis which is associated with poor patient prognosis. MCAM contributes to melanoma progression by regulating cell adhesion, promoting cell migration, increasing invasion, and stimulating angiogenesis. The recycling of MCAM through the endocytic network is potentially an important factor in melanoma metastasis, and preliminary work has identified two putative endocytosis/recycling motifs in the intracellular domain of MCAM. To further this investigation, the MCAM-negative melanoma Colo239F cell line was transduced to express wild-type MCAM as well as MCAM containing disruptive mutations in these motifs. Characterisation of these cell lines has produced results consistent with previous studies conducted in our laboratory. The cell lines display subcellular localisation and cell surface expression of MCAM consistent with other melanoma cell lines. Interestingly, preliminary work suggests a dileucine endocytosis motif may be involved in the polarised targeting of MCAM to the rear of the cell, a mechanism thought to contribute to cell migration. Furthermore, retromer, an evolutionarily conserved protein complex and key player in endosomal sorting, may be involved in MCAM recycling. Analysis of core retromer components and sorting nexin expression levels suggest that these components are upregulated in metastatic melanoma, except sorting nexins 17 and 27, which appear to be downregulated. MCAM has long been investigated as a potential therapeutic target and elucidating the role and mechanism of MCAM recycling in melanoma metastasis may lead to advancements in this area.

E4

Augmenting Neoantigen Vaccination with Immunogenic Chemotherapy

Jessica Boulter[1,2], Jenette Creaney[2], Bruce Robinson[1,2], Alec Redwood[2]

[1]The University of Western Australia, [2]National Centre for Asbestos Related Diseases

Introduction. Mutations in tumour cells give rise to neoantigens, fragments of which are presented on the tumour cell surface. Neoantigens are exclusively expressed by tumour cells, making them favourable targets for cancer immunotherapy. Early clinical trials indicate that vaccines targeting neoantigens are insufficient at inducing protective anti-tumour immunity alone[1]a class of HLA-bound peptides that arise from tumour-specific mutations. They are highly immunogenic because they are not present in normal tissues and hence bypass central thymic tolerance. Although neoantigens were long-envisioned as optimal targets for an anti-tumour immune response, their systematic discovery and evaluation only became feasible with the recent availability of massively parallel sequencing for detection of all coding mutations within tumours, and of machine learning approaches to reliably predict those mutated peptides with high-affinity binding of autologous human leukocyte antigen (HLA. Gemcitabine, a chemotherapy drug that exerts immunomodulatory effects[2]solid tumors to CD8 cells is efficient and likely to have a role in determining host response to tumor. A number of investigators have predicted that when tumor Ags are derived from apoptotic cells either no response, due to Ag \"sequestration,\" or CD8 cross-tolerance would ensue. Because the crucial issue of whether this happens in vivo has never been addressed, we induced apoptosis of established hemagglutinin (HA, may increase the number of immunologically recognised neoantigens and synergise with neoantigen vaccines to eliminate tumours. **Problem Statement**. Gemcitabine will increase the number of immunologically recognised candidate neoantigens and will synergise with neoantigen vaccines to delay tumour growth in a murine model of mesothelioma. **Procedures**. BALB/c mice were inoculated with AB1-HA tumour cells. When tumours measured 16-20mm^2, mice received either a single dose of gemcitabine (n=5) or vehicle (n=5). 7 days later mice were euthanised and lymphocytes were isolated from the tumour-draining lymph nodes (LNs) and spleen. ELISpot was used to measure interferon-gamma (IFN-γ) production by lymphocytes in response to synthetic peptides that were predicted to be candidate neoantigens. Spot-forming units were used to quantify the number of cells producing IFN-γ. **Results.** Immunological recognition of candidate peptides in four peptide pools was observed in the combined LN lymphocytes of both treated and untreated mice. Response to the same four pools was observed in the spleen of a single untreated mouse (1/3). Deconvolution of these pools revealed that two peptides are known neoantigens, and two are novel candidates. **Conclusions**. Gemcitabine does not increase the number of immunologically recognised candidate neoantigens in AB1-HA tumours, however two novel neoantigens may have been identified. Further verification of these results will be carried out using IFN-γ ELISpot and intracellular staining. If confirmed, these neoantigens may prove beneficial in a neoantigen vaccine in a pre-clinical model, which can be used to inform further research into neoantigen vaccines.

E5

Deciphering the Secretory Lysosome 'Transportome' Unveils Novel Regulators of Osteoclast Function and Bone Homeostasis

Amy B.P. Ribet[1], Daniel Yagoub[1], Laila N. Abudulai[1,2], Pei Ying Ng[1] and Nathan J. Pavlos[1]

[1]School of Biomedical Sciences, The University of Western Australia, Perth , [2]Centre for Microscopy, Analysis and Characterisation and School of Molecular Sciences, The University of Western Australia

The ruffled border is a lysosome-related, bone-digesting organelle unique to osteoclasts. It is formed upon the fusion of secretory lysosomes with the bone-apposed plasma membrane. Fusion of secretory lysosomes with the ruffled border releases cathepsin k into the underlying resorptive space. At the same time, it equips the ruffled border with sets of lysosomal membrane proteins that are requisite for bone resorptive function. Despite its crucial importance, our understanding of the ruffled border's molecular anatomy and its secretory lysosomal progenitors remains limited. In particular, we still lack elementary information on the protein composition of the ruffled border, including the numbers and identities of lysosomal membrane residents whose usual functions are to facilitate the exchange of molecules across its membrane (i.e. transporters). To extend the molecular inventory of transporters operating at the ruffled border, we have combined biochemical methods with high-resolution tandem mass spectrometry (LC-MS/MS) to unbiasedly survey the osteoclast lysosomal membrane proteome using isolated secretory lysosomes as a surrogate. Using this approach, we unambiguously identified 2351 unique proteins, of which 421 were enriched on secretory lysosomes, including 102 unique IDs functionally assigned as membrane transporters. These transporters include all subunits of the V-ATPase proton pumps, chloride ion channels, secondary active transporters of the Solute carrier (Slc) protein superfamily, and others whose localisations are predicted to reside on lysosomes, but their functions remain unknown. By combining a suite of biochemical, cell biology and genetic studies, we demonstrated the robustness and utility of our proteomic screen using the Slc37a2 transporter as a prototype. This approach has allowed us, for the first time, to unmask the entire osteoclast lysosomal transporter cache (termed the 'Transportome'), that will serve as a powerful resource for the future interrogation of 'orphan' lysosomal transporters, whose physiological functions in osteoclasts and bone homeostasis have yet to be ascribed.

E6

Is a PITP a novel regulator of encystation in *Giardia duodenalis*?

Aleesha Davis[1,2], Dr. Carl Mousley[1,2], Dr. Rob Steuart[2]

[1]School of Pharmacy and Biomedical Sciences, [2]Curtin Health Innovation Research Institute, Curtin University, Perth, WA

Giardia duodenalis is an intestinal parasite with an estimated 280 million symptomatic cases annually worldwide. *Giardia* has a simple two step life-cycle; the trophozoite and the environmentally resistant cyst. The cyst is produced through the process of encystation. This process involves the production, synthesis and secretion of cyst wall proteins (CWPs) forming an extracellular matrix, conferring environmental resistance to the cyst. The trafficking of these CWPs via encystation specific vesicles (ESVs) is the only known regulated export pathway in *G. duodenalis*. Whilst *G. duodenalis* has no known Golgi apparatus, ESVs are considered to perform a similar function. Whole cell proteomic analysis has identified the expression of a putative phosphatidylinositol transfer protein (PITP) to be elevated during encystation, however not much is known about PITP function in *G. duodenalis*, through studies in other eukaryotes indicated that this expression profile is not coincidental. It is hypothesized that *Gd* PITP dependent PtdIns-4-P production is essential to promote trafficking of CWPs to the cell periphery during the developmental progression from the trophozoite to the environmentally resistant cyst. Preliminary data from our lab determined the increased gene expression of *Gd* PITP during encystation. Additionally an expression profile has been generated using a heterologous expression system for PITP and *Gd* PITP is found to be toxic to numerous mutants defective in PtdInd-4-P synthesis and the phenotypes are reminiscent of that determined for a unique PITP, sfh3. Furthermore a system has been generated to determine the lipid binding activity of *Gd* PITP to further investigate the function of this protein. As little is known about the molecular mechanisms that control encystation in G. duodenalis, understanding these mechanisms may act as a potential target for pharmaceutical intervention, diminishing the ability of *G. duodenalis* to successfully complete encystation, therefore ablating environmental resistance.

Lactoferrin Promotes House Dust Mite Protease-Induced Gut Damage: Implications for Allergic Disease Development

Byrne J, Lai CT, Attwood P, Verhasselt V, Macchiaverni P

School of Molecular Sciences, University of Western Australia

Dermatophagoide pteronyssinus (*Der p*) allergens are found in human breastmilk and their presence is associated with increased respiratory and food allergy risk in breastfed children. Data gathered in mice showed that *Der p* proteases play a key role in this increased risk, by dysregulating gut epithelial barrier function. Modulating *Der p* protease activity is therefore a promising strategy to counteract allergy development. In this study, lactoferrin (LF), a poly-functional glycoprotein largely present in breastmilk, was investigated as a candidate protease inhibitor of *Der p*. Methods: *Der p* crude extract was incubated with LF purified from human milk and enzymatic assay was used to analyse changes in protease activity. Human intestinal CaCo2 cells were used to investigate whether LF could mitigate *Der p* associated increase in gut permeability, as assessed by changes in transepithelial electrical resistance (TEER). Alteration in the expression of tight junction protein Zona-Occludens 1 (ZO-1) in CaCo2 cells monolayers was examined by immuno-fluorescent staining. Results: Kinetic studies revealed that LF increased, rather than decreased, *Der p* protease activity. The addition of LF to *Der p* increased the maximum velocity [mean V_{max} (CI 95%) from 1.02 (0.96-1.07) to 1.104 (1.053-1.14)]. Moreover, CaCo2 cell monolayers exposed to only *Der p* experienced a 29.7% reduction in TEER, while a 53.2% decrease was observed when treated with both *Der p* and LF. Finally, we found that the addition of *Der p* to CaCo2 cells disrupted the expression of ZO-1 in tight junctions and LF worsened this effect. Conclusion: These *in vitro* data show that LF increases *Der p* protease activity and associated gut epithelium damage. If confirmed by *in vivo* experiments, these data would indicate that LF can favour allergy development, highlighting the importance of finding ways to mitigate these deleterious effects to decrease allergy risk in children.

Evidence of Rare T-cell Population Expansion in Inclusion Body Myositis

Emily Thomas[1], Jose Castro Suarez,[1] Kelly Beer[1,2], Merrilee Needham (MBBS, FRACH, PhD)[1,2,3,4], Jerome Coudert (PhD)[1,2,3]

[1]Institute for Immunology and Infectious Disease, Murdoch University; [2]Centre for Molecular Medicine and Innovative Therapeutics, Perron Institute for Neurological and Translational Science; [3]University of Notre Dame Australia; [4]Fiona Stanley Hospital; Western Australia

Inclusion body myositis (IBM) is an inflammatory myopathy that most frequently affects patients over 45, causing atrophy and weakness in proximal and distal muscles. Recently, expansion of rare T cells which includes $CD8^+$ T cell large granular lymphocytes (TLGL) and gamma-delta (γδ) T cells was revealed. T-LGL expansion is a precursor of T-LGL leukaemia and presents an additional burden for IBM patients. γδ-T cells constitute an unconventional T cell subset that exhibits anti-microbial and anti-tumour functions. The pathoetiology of IBM is still poorly understood, however given the expansion of these cells in IBM patients, we can hypothesise that they play an important role in the disease. We aim to confirm if T-LGL leukaemia and expanded γδ-T cells are associated with IBM. Peripheral blood samples taken from IBM patients and healthy donors were analysed using flow cytometry. We found evidence of T-LGL expansion in 7/19 (37%) of IBM patients, compared to 0% of healthy controls (H.C). T-LGL populations shows a cytotoxic, late-differentiated $CD8^+$ T cell phenotype characterized by $CD57^+$, $CD5^{dim/low}$, $CD94^+$, $KLRG1^+$. 1/3 of IBM patients show a higher percentage of γδ T cells compared to H.C (ranging between 4-6% vs Mean ± SEM = 1.298 ± 0.3421% in H.C) with a noticeable shift in their T cell receptor (TCR) repertoire, predominated by Vδ1 in IBM rather than Vδ2 in H.C. $V\delta1^+$ display a TEMRA phenotype ($CD45RA^+$, $CCR7^-$) characteristic of late-differentiation and increased expression of the proliferation-associated Ki67 in $V\delta1^+$ in IBM compared to H.C (P= 0.0173). Our results demonstrate that two populations of rare T cells expand in IBM patients but not H.C, indicating that both T-LGL and γδ T cells are associated with the disease and possibly contribute to its pathoetiology. Further investigations are needed to gain further insights into the phenotype and role of these populations.

F3

Characterising Immune Infiltrates After Intratumoural Poly(I:C) Treatment

Breana Weston[1,2], Francois Rwandamuriye[2,3], Rachael Zemek[2], Joost Lesterhuis[2,3]

[1]Murdoch University, College of Science, Health, Engineering and Education, Murdoch, Australia, [2]Telethon Kids Institute, Perth, Australia, [3]University of Western Australia, Perth, Australia

Poly(I:C) is a synthetic double stranded RNA analogue which has been used to mimic viral infections and as a vaccine adjuvant. It binds to Toll-like receptor 3 (TLR3) which is expressed by both immune cells and non-immune cells. Once activated by poly(I:C), TLR3 signals downstream to produce type I interferons. Previous studies have shown that the use of poly(I:C) as an adjuvant in cancer therapy can effectively reduce tumour growth by directly causing cancer cells to undergo apoptosis, immune-mediated cancer killing, and T cell-mediated immune memory. Acting similar to a vaccination, intratumoural poly(I:C) could increase tumour regression in many patients, including those who have metastatic disease or have not responded to other treatments. Before this potential immunotherapy can be used in cancer patients, it is important to understand the effects within the tumour. This project aims to identify which immune cells infiltrate the tumour microenvironment in response to poly(I:C) immunotherapy. To do this we used tumour-bearing mice treated with intratumoural poly(I:C) for three days, and analysed dissociated tumours at different time points using flow cytometry. This technique allowed us to identify the tumour-infiltrating immune cell populations and how they changed over time following poly(I:C) treatment. By identifying the association between the changes in tumour-infiltrating immune cell populations and poly(I:C) treatment it is possible to target further research to understand how poly(I:C) can be used to treat cancer.

F4

Immune Disturbances in Dysferlin-deficient Spleens of BlA/J Mice.

Mojiroluwa O Oyemade[1], Miranda D Grounds[1], Connie Jackaman[2], Erin M Lloyd[1]

[1]School of Human Sciences, the University of Western Australia, Perth, Australia; [2] Curtin Health Innovation Research Institute, Curtin University, Perth, Australia

Introduction. Dysferlinopathies are muscular dystrophies caused by mutations in the DYSF gene, leading to absence of dysferlin (a membrane associated protein) in many cells, including skeletal muscle, macrophages and neutrophils. Dysferlinopathy manifests post-puberty and is characterised by progressive muscle wasting with adipose replacement of muscle tissue; the precise mechanism for the dystropathology is unknown and there is no specific treatment. The disease is associated with exaggerated inflammation in muscles, and enlarged spleens with disturbed macroscopic appearance were noted in very old dysferlin-deficient ($dysf^{-/-}$) BLAJ mice aged 26 months. Since the immune system becomes dysregulated during normal healthy ageing (immunoageing), this may influence manifestation in dysferlinopathy. **Problem.** To investigate the impact of dysferlin-deficiency on the immune system, spleens from $dysf^{-/-}$ BLAJ and control wild type (WT) C57Bl/6J mice were analysed at 3 ages: 3, 10 and 26 months. **Procedures**. Spleens were obtained from all mice to assess microanatomy and cell composition using immunostained frozen tissue sections to identify populations of neutrophils, macrophages and T lymphocytes in tissue. **Results.** For female mice aged 26 months, there was a striking increase (>2 fold) in BLAJ spleen mass with high variation (mean ±SEM) of 296±95.24 mg ($p<0.05$ for n=5 mice), compared with WT spleen mass of 123.8 ±12.04 mg (n=5); this difference was not evident in younger mice. The old BLAJ spleens had very disturbed microanatomy with enlarged red and white pulp zones, indistinct zone boundaries and neutrophil infiltration into white pulp. **Conclusions**. The old dysferlinopathic spleens show significant immune system dysregulation; these novel data provide deeper insight into the pathophysiology of dysferlinopathies, and may provide new targets for drug based therapies for this human disease.

F5

Decline in magnitude of ZIKV-specific memory T-cells in monotypic and DENV / YFV-17D exposed individuals

Hannah Greig[1], Laila Abudulai[1,2,3], Allison Imrie[1,4], David Smith[1,4]

[1]School of Biomedical Science, The University of Western Australia, [2]Centre for Microscopy, Characterisation and Analysis, The University of Western Australia,[3]School of Molecular Sciences, The University of Western Australia [4]PathWest Laboratory Medicine, QEII Medical Centre

Longer duration of pre-existing dengue virus (DENV) immunity is associated with severe disease outcomes. Long-term DENV-specific T-cell immunity is associated with decline in proliferative and cytokine responses 3-9 years after infection. Prior DENV exposure is increasingly reported to influence Zika virus (ZIKV)-specific immunity and that ZIKV-specific T-cells may recognise DENV peptides, likely mediated by sequence similarities between the two viruses. This cross-reactivity has only been documented in the first month post infection (MPI), it is unknown if ZIKV-specific T-cells maintain DENV cross-reactive potential and similar cytokine and cytolytic responses months to years after infection. **Method:** Using INFγ ELISpot and ZIKV PRVABC59-based peptides we measured ZIKV-specific T-cell responses in 8 individuals with monotypic ZIKV infection or ZIKV infection with other flavivirus exposure, sampled between 9 and 42 MPI. Sequential samples were analysed in 4 individuals. **Results** A decline in magnitude of the ZIKV-specific IFNγ response was seen between 9 and 20 MPI (3 individuals) and 30 and 37 MPI (1 individual). A reduction in breadth of responses to ZIKV peptides was noted in 2 of 4 individuals. ZIKV-infected subjects with prior flavivirus exposure had higher magnitude responses to ZIKV peptides compared with monotypic subjects, however the difference was not significant. $Capsid_{21-35}$, was recognised in 3/3 individuals carrying the A*03:01 allele, and 1/2 individuals? with the A*66:01 or A33*01 alleles. **Conclusion:** The frequency of ZIKV-specific IFNy-producing T-cells declines over time in the absence of re-exposure. Future studies, will examine the memory phenotype, cytokine profile and cross-reactive potential of ZIKV-specific memory T-cell and this will better inform our understanding of the long-term ZIKV-specific memory response.

F6

Polymicrobial Infection and Neutrophilic Disease in Cystic Fibrosis Airways

Daniel R. Laucirica[1,2], Craig J. Schofield[1], Samantha A. McLean[1], Camilla Margaroli[3,4], Stephen M. Stick[1,2,5], Rabindra Tirouvanziam[3,4], Anthony Kicic[1,2,5,6], Luke W. Garratt[1,2], on behalf of AREST CF[1,5,7,8]

[1]Telethon Kids Institute [2]Medical Sciences, Univ. of WA [3]Emory Univ., USA [4]Children's Healthcare of Atlanta, USA [5]Dept. of Respiratory and Sleep Med., PCH [6]School of Public Health, Curtin Univ. [7]Murdoch Children's Research Institute [8]Dept. of Paediatrics, Univ. of Melbourne

Cystic fibrosis (CF) lung disease is driven by a cycle of infection, powerful innate immune responses, and neutrophilic inflammation. Neutrophils in CF airways are reprogrammed to an inflammatory phenotype, as shown by a decrease in functional marker CD16 (reduced phagocytosis) and an increase in marker CD63 (hyperexocytosis of proteolytic granules), however factors that induce reprogramming are poorly understood. We created a multi-component *in vitro* system to model CF inflammatory responses and airway neutrophil recruitment, specifically in the context of polymicrobial airway infection. This was used to assess epithelial and neutrophil responses to rhinovirus and *Pseudomonas aeruginosa* infection. Submerged monolayers of CF and non-CF primary airway epithelial cells (pAEC) were infected individually and in combination with rhinovirus strain RV1b (MOI 0.5) and a mucoid P. aeruginosa clinical isolate (MOI 0.001). After 48 hours, infection supernatants were harvested and epithelial cytokines quantified by ELISA. Filtered supernatants were also applied to an *in vitro* model of neutrophil transmigration to the airways. Neutrophils were migrated for 10 hours, harvested, and assessed by flow cytometry. Both infection with RV1b or RV1b+*P. aeruginosa* significantly increased production of proinflammatory cytokines IL-8, IL-1β, and TNFα in both CF and non-CF pAEC compared to uninfected controls or bacterial infection alone. Neutrophils migrating towards CF co-infection supernatants had significantly reduced staining of CD16 ($p<0.01$) and increased staining of CD63 ($p<0.02$), indicative of reprogramming. Neutrophils migrating towards non-CF supernatants were not affected in a significant manner. Results highlight the role of respiratory viral infections as triggers of airway inflammation. CF coinfection supernatants induced the greatest shifts towards aberrant neutrophil phenotypes, suggesting that polymicrobial infections may be implicated in CF neutrophil reprogramming. This model permits investigation of CF airway responses to diverse pathogenic insults and the mechanisms that drive airway inflammation.

F7

Use of Confocal Microscopy to Identify Epstein-Barr Virus in B-cell Subsets

Sithu Aung[1,2], Jordana Sheahan[1], Dorothy Luk[1], Dr Kajia Strautins[1], Associate Prof Alec Redwood[1], Associate Prof David Nolan[1,3], Dr Monika Tschochner[1,2]

[1]Institute of Immunology & Infectious Disease, Murdoch University, [2] University of Notre Dame Australia, [3]Royal Perth Hospital

Introduction: Epstein-Barr virus (EBV), is a ubiquitous γ-herpesvirus that establishes life-long infection in approximately ~90% of adults. EBV infection can result in infectious mononucleosis, and has been linked to Multiple Sclerosis. Epstein-Barr Nuclear Antigen-1 (EBNA-1) and Epstein-Barr virus-encoded small RNAs (EBER), are two important viral products, constitutively expressed in latency. **Aim:** This study aimed to visualize EBV in B-cells in order to identify which B cell subset/s are infected by using confocal microscopy in conjunction with EBV specific smartflare™ technology. **Method:** Smartflares targeting EBNA-1 and EBER via complementary RNA coupled flourescent nanoparticles were tested using 2 healthy human controls and 1 EBV-transformed cell-line. Samples were previously confirmed for EBV seropositivity using ELISA, while EBV copy number was quantified using qPCR. Healthy controls were B-cell enriched by depleting $CD4^+$ and $CD8^+$ T-cells. Cells were subsequently incubated with SmartFlares for 16hrs, then washed, fixed to coverslips, and stained with fluorescent antibodies targeting CD19, CD20, IgG and CD27 using the Nikon $C2^+$ Confocal microscope. **Results:** Smartflares were shown to bind to both EBER and EBNA-1 successfully usinga cell-line. Latently infected healthy control samples were smartflare positive in high copy number only. In the positive sample, a total of 80 cells were smartflare positive, of which 73 were EBNA-1 positive memory B-cells, expressing both CD20, and CD27. Additionally, 3 more memory B-cells were positive for EBNA-1, IgG, and CD27. Only 5 cells were identified to be positive for both EBNA-1 and EBER smartflares. **Discussion:** From the results, these Smartflares have provided the evidence for proof of principle that the technology can be used to identify EBV infection in B cell subsets. Future analysis should focus on larger sample size and compare results to MS patient samples in order to develop targeted treatment in Multiple Sclerosis patients in the future.

F8

Adjuvant Activities Of Type I Interferons

Audsley K[1], Wagner T[1], Ta C[1], Buzzai A[1], Newnes H[1], Foley B[1], and Waithman J[1]

[1]Telethon Kids Institute, The University of Western Australia, Australia

Immunotherapies such as checkpoint blockade therapy are revolutionising outcomes for cancer patients. However, further advances are required for the majority of patients to benefit. One reason patients may not respond to checkpoint blockade is due to a suboptimal anti-tumour T cell response. Anti-cancer vaccines are currently being employed to generate robust tumour-specific T cell immunity. A key ingredient for effective vaccination is the inclusion of an appropriate adjuvant that boosts anti-cancer responses, potentially sensitising patients to checkpoint blockade. We investigated the adjuvant potential of individual members of the type I interferon (IFN) cytokine family using a melanoma whole-cell vaccine approach. To this end, mice received irradiated B16 melanoma cells expressing a single IFN subtype. We have identified that several subtypes demonstrate superior adjuvant potential as compared to the current gold standard adjuvant, polyI:C. In particular, IFNβ induced significantly greater priming and activation of systemic tumour-specific $CD8^+$ T cell responses than polyI:C and all other IFN subtypes tested. This $CD8^+$ T cell expansion is dependent on the presence of $CD4^+$ T cells, CD40/CD40L signalling, and $XCR1^+$ dendritic cells (DCs). Increased tumour-specific T cell infiltration into the tumour microenvironment was observed with IFNβ, with infiltrating $CD8^+$ T cells upregulating PD1 expression. Combination of our whole-cell vaccination strategy with anti-PDL1 checkpoint blockade treatment significantly delayed tumour growth. Thus, vaccination strategies incorporating IFNβ in combination with anti-PDL1 therapy has the potential to promote strong immune responses and improved therapeutic outcomes for patients.

F9

Divergent Retention of Donor Myeloid Cells in Liver Transplantation

Sarah Dart[1], Amy Prosser[1], Wen Hua Huang[1], Liu Liu[1], Monalyssa Watson[1], Bastiaan de Boer[2], Gary Jeffrey[1], Luc Delriviere[1,3], Axel Kallies[4], Michaela Lucas[1,5]

[1]University of Western Australia; [2]Department of Anatomical Pathology, Fiona Stanley Hospital; [3]Western Australian Liver and Kidney Transplant Service, Sir Charles Gairdner Hospital; [4]University of Melbourne; [5]Department of Immunology, Sir Charles Gairdner Hospital.

Introduction. Solid organ transplantation involves the transfer of donor immune cells simultaneously, and donor myeloid cells are thought to play an important role in solid organ transplantation outcome. However, the destiny of donor myeloid cell subsets following transplantation has not been investigated. **Problem Statement**. We hypothesised that donor myeloid cell subsets would be differentially retained in response to liver transplantation in mice. **Procedures**. Orthotopic liver transplants between genetically matched (congenic) and mismatched (allogeneic) mice were performed. To distinguish donor and recipient monocytes, donor and recipient strains with differential expression of leucocyte marker CD45 (donor, CD45.1; recipient, CD45.2) were used. Donor and recipient macrophages, monocytes, dendritic cells and neutrophils in the liver, spleen, lymph nodes, bone marrow and blood were analysed by flow cytometry at day 0, 1, 7 and 28 post-transplantation. **Results**. Following transplantation, the total number of donor myeloid cells in the liver was rapidly decreased in both models by day 7 (congenic, 20% of their baseline number; allogeneic, entirely depleted). In the congenic model, of the donor myeloid cells remaining in the liver at day 28, approximately 70% were monocytes. Donor myeloid cells were detected in the spleen, bone marrow and lymph nodes as early as day 1 post-transplantation in the congenic model, but undetectable in the allogeneic model. Recipient myeloid cells, predominately macrophages, infiltrate the graft liver following transplantation in both models, making up >98% of total liver myeloid cells by day 7. **Conclusions**. Following liver transplantation, donor myeloid cells are differentially retained in genetically matched, but depleted in mismatched, mice. Simultaneously, donor myeloid cells are almost entirely replaced by recipient cells. These findings may be significant in relation to antigen presentation, infection control and rejection responses. Further investigations into the effect of variations in the level of mismatch on myeloid cell populations are underway.

F10

Development of a Mouse Model of Incompletely Resected Soft Tissue Sarcoma for Testing Novel Perioperative Treatments to Prevent Recurrence

Francois Rwandamuriye[1], Breana Weston[2], W. Joost Lesterhuis[1,2], Rachael Zemek[2]

[1]University of Western Australia, School of Biomedical Sciences, Perth, Australia; [2]Telethon Kids Institute, Perth, Australia

Soft tissue sarcoma is a cancer that develops from the mesenchymal tissue. Although soft tissue sarcomas are rare tumours in adults, they account for up to 20% of all paediatric cancers. Surgery is the main treatment option, usually in combination with chemotherapy or radiotherapy. Yet, there is still a high risk of local relapse and subsequent metastasis after surgery. Immunotherapy with checkpoint blockade, such as antibodies against programmed cell death protein 1 (anti-PD-1) is largely ineffective. Therefore, there is an urgent need for new strategies to tackle the problem of cancer relapse in soft tissue sarcomas. Given the potential of wound healing responses negatively affecting the local immune milieu, preclinical models should incorporate (partial) surgical resection when testing new treatment strategies. Here, we aimed to develop a mouse model of partial surgical debulking of soft tissue sarcomas in order to test immunotherapies peri-operatively. To do this, fibrosarcoma cell line WEHI-164 was inoculated subcutaneously in BALB/c mice and primary tumours were partially (50 or 80%) debulked when tumours were approximately 50 mm^2 in size. Checkpoint blockade antibodies anti-PD-1 or anti-CTLA4 were dosed intraperitoneally post-operatively. Efficacy of immunotherapy was assessed by measuring tumour regression and analysis of survival curves. Safety was assessed by assessing the wound healing macroscopically. Incomplete surgical resection resulted in reproducible regrowth of the tumour in all mice in the absence of adjuvant immunotherapy. Use of immune checkpoint blockade antibodies after surgery resulted in a cure rate of 25% with anti-PD-1. In conclusion, we developed a mouse model for incomplete surgical resection of soft tissue sarcomas. This will allow preclinical testing of (neo)adjuvant therapies in soft tissue sarcoma, both alone or in combination with anti-PD-1 therapies. This model can be applied to other types of solid cancers.

F11

Capture of HCV E2-Specific Antibody Sequences from Patients

Sahar Safaeefar[1], Murray Porter[1], Silvia Lee[2], Mark Watson[3], Jane Allan[1]

[1]School of Biomedical Sciences, University of Western Australia, [2]Harry Perkins Institute of Medical Research, [3]Institute for Immunology and Infectious Diseases, Murdoch University,

Hepatitis C virus (HCV) remains the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. The rapid generation of cross-reactive, broadly neutralising antibodies against HCV E2 glycoprotein has been associated with protection from chronic HCV infection. However, *in vitro* characterisation of such antibodies is limited. The aim of this project was to devise a simple method to isolate E2-specific memory B cells from HCV-infected patients through stimulation with E2 coated magnetic IMAC beads. This new procedure uses 6x his-tagged E2 glycoprotein bound to magnetic IMAC beads to identify memory B cells from HCV antibody-positive patients. Culture supernatant from HEK293 cells stably secreting 6x his-tagged E2 glycoprotein (genotype 1b) was collected every 2 days for 12 days to identify the optimal harvest time. E2 was purified using Ni-IDA resin and bound to an ELISA plate by GNA lectin for detection using anti-6x his-tag antibody. E2 was found from day 4 and day 6 and selected for harvest. The production of E2 was confirmed by a molecular weight, 55kDa peak on a gel, and by reactivity in ELISA with anti-E2 monoclonal antibody. The quantity of purified, concentrated E2 required to saturate the IMAC beads was then determined. E2 glycoprotein coated beads, and beads without E2, were incubated with CD19+ B cells isolated by negative selection from white blood cells of HCV-infected patients. IgG producing cells were detected using AF488 conjugated goat $F(ab)_2$ anti-human IgG Fcg, and picked by micromanipulation. Following RT-PCR, the sequence of the variable region of the B cell receptor will be determined and cloned into an expression system to produce anti-E2 antibodies or FAB fragments. If successful, this study will provide a new method of isolating E2-specific memory B cells for antibody production, bringing vaccine design a step closer.

F12

Immune system characterization in Inclusion Body Myositis (IBM)

Jose A. Castro Suarez[1], Emily Thomas[1], Kelly Beer[1,2], Merrilee Needham (MBBS, FRACP, PhD)[1,2,3,4], Jerome Coudert (PhD)[1,2,3]

[1]Institute for Immunology and Infectious Disease, Murdoch University; [2]Centre for Molecular Medicine and Innovative Therapeutics, Perron Institute for Neurological and Translational Science; [3]University of Notre Dame Australia; [4]Fiona Stanley Hospital; Western Australia

Introduction. Inclusion Body Myositis (IBM) is an inflammatory, autoimmune disease that targets skeletal muscles, causing progressive degeneration and loss of function that impacts quality of life. The underlying pathological mechanisms are not understood yet and no efficient cure has been discovered; current immunosuppressive treatments provide little to no benefits. By employing a cross-sectional study, we can characterize the autoimmune manifestations, and identify the altered leukocytic populations associated with IBM. This project has the potential to provide the basis for the development of better diagnostics through the identification of biomarkers and more targeted therapies. **Problem Statement**. We hypothesised that the autoimmune manifestations associated with IBM are detectable on the immune cells. These manifestations might be correlated to IBM severity and its progression. **Procedures**. Peripheral blood obtained from IBM patients as well as aged-matched healthy donors was labelled using antibodies and analysed by flow cytometry to identify the leukocytic populations (i.e. T-cells, B-cells, etc…). We also investigated cell proliferation by analysing Ki67 expression within the cell subsets. **Results**. To date, our data indicate an increased frequency of the inflammatory macrophage subset in IBM patients compared to healthy controls. Also in IBM, most T cells (both $CD4^+$ and $CD8^+$) display activated/memory features and contain cytotoxic and inflammatory molecules. Likewise, B lymphocytes are mostly activated in IBM patients. These activated inflammatory cells are currently being further investigated for Ki67 content; this will indicate which particular subsets are most actively proliferating. **Conclusions**. Characterizing the immune response in IBM will contribute to the overall understanding of the disease and its underlying mechanisms. Understanding the changes affecting the immune system in IBM will point to future directions to investigate more precisely in the future, with the objective to achieve more targeted therapies.

Rogue Antibodies and How to Stop Them: Aptamer Neutralisation of Pathogenic Autoantibodies in Inclusion Body Myositis.

Nataliya Slater[1], Tao Wang (PhD)[2, 3], Kelly Beer[1, 2], Christine Bundell (PhD)[4], Merrilee Needham (MBBS, FRACH, PhD)[1, 2, 3, 5], Rakesh N Veedu (PhD, MRACI)[2], Jerome Coudert (PhD)[1, 2, 3]

[1]Institute for Immunology and Infectious Diseases, Murdoch University; [2]Centre for Molecular Medicine and Innovative Therapeutics, Perron Institute for Neurological and Translational Science; [3]University of Notre Dame; [4]PathWest; [5]Fiona Stanley Hospital; Western Australia

Introduction. Inclusion Body Myositis (IBM) is the most commonly diagnosed inflammatory muscle disease associated with aging. It is characterized by degeneration of skeletal muscles that inevitably leads to the loss of mobility. The definitive cause of IBM remains unclear, and there is currently no effective treatment. Recent studies revealed circulating autoantibodies that target cytosolic 5'-nucleotidase 1A (cN1A) - an enzyme that is highly expressed in muscles. P**roblem Statement**. Anti-cN1A antibodies may play a role in the pathogenic process of IBM; thus, therapeutic interference with their binding may have a favourable effect on the course of the disease. **Procedures**. We have screened the sera of 42 IBM patients by enzyme-linked immunosorbent assay (ELISA) and selected a donor with high concentration of autoantibodies. The antibodies were purified out of the serum using magnetic beads conjugated to histidine-tagged cN1A protein. We have randomly generated a library containing 10^{15} segments of ~80-nucleotide-long single-stranded DNA (aptamers). This library was screened against the purified antibodies to select the aptamers that form conjugates with the antibodies' binding domain and by doing so prevent antibody binding to cN1A. The retained aptamers were PCR-amplified and are currently further screened; approximately 10 cycles of this process will be required to isolate the highest affinity binders. **Results**. To date, we have confirmed the presence of anti-cN1A antibodies in 45% of IBM patients' sera. The antibodies were isolated and concentrated to 1.9 mg/mL as measured by the bicinchoninic acid (BCA) protein assay; ELISA confirmed that the binding of these antibodies to cN1A had not been affected through this process. We have completed the first round of aptamer screening and are proceeding through the selection process. **Conclusions**. The results of this project may contribute to the development of novel treatments that limit the disease progression or possibly revert the symptoms in IBM patients.

Morpholino Oligomer-Induced Dystrophin Isoforms: Mapping the Functional Domains in the Distal Third of the Dystrophin Gene

Dunhui Li[1,2], Abbie Adams[1,2], Russell Johnsen[1,2], Sue Fletcher[1,2], Steve Wilton[1,2]

[1]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, [2] Perron Institute for Neurological and Translational Science

Introduction. The dystrophin protein plays a crucial role in maintaining sarcolemma stability during muscle contractions, and mutations which prevent the expression of a functional protein result in the progressive muscle wasting disorder, Duchenne muscular dystrophy (DMD). Antisense oligonucleotide-mediated manipulation of pre-messenger RNA splicing to by-pass DMD-causing mutations and restore some functional dystrophin expression has now entered the clinic for the most common type of DMD mutations: frameshifting genomic deletions flanking exons 45 (Casimersen, Phase 3 trials), 51 (Eteplirsen, conditional approval) and 53 (Golodirsen: under FDA evaluation). **Problem Statement**. This strategy is based upon the genotype-phenotype correlations observed in the cases of Becker muscular dystrophy (BMD), where in-frame deletions of some of the dystrophin exons, especially in the central rod domain, result in internally truncated but semi-functional dystrophin protein isoforms. However, genomic deletions in the latter third of the dystrophin gene are very rare, and consequently BMD genotype-phenotype correlations cannot be made. Consequently, the amenability of mutations in the latter third of the DMD to exon skipping strategies remains unknown. **Procedures**. In this study, dystrophin "BMD-like" isoforms were induced in vivo in mice after intraperitoneal injection of the peptide-conjugated phosphorodiamidate morpholino oligomers to skip in-frame exon blocks of 56+57 and 58+59. **Results**. The isoform lacking exons 56+57 appears to be more functional than the isoform without exons 58+59, which was demonstrated by increased dystrophin expression and stabilized β-dystroglycan. **Conclusions**. Less muscle degeneration, less connective tissue infiltration and more normal looking muscle were observed in mice after the induction of the dystrophin isoform missing exons 56+57, indicating some functionality and therapeutic potential for DMD-causing mutations in these exons.

Investigating Molecular Therapeutics for Amyotrophic Lateral Sclerosis

Rita Mejzini[1, 2], Loren Flynn[1, 2], Sue Fletcher[1, 2], Steve D Wilton[1, 2] Anthony Akkari[1, 2],

[1]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, [2]The Perron Institute for Neurological and Translational Science, Perth

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease characterised by degenerative changes to both upper and lower motor neurons. It usually presents as a relentlessly progressive muscle atrophy and weakness, with the effects on respiratory muscles limiting survival to 2 to 4 years after disease onset in most cases. Current treatment options are based on symptom management and respiratory support, with the only approved medications prolonging survival for just a few months. A common feature in ALS is the cytoplasmic aggregation of proteins. Mutations in several ALS genes (including SOD1, FUS, TARDBP and C9ORF72) are known to lead to the pathologic aggregation of their encoded proteins. The transactive response DNA-binding protein 43 (TDP-43) encoded by the TARDBP gene was identified in 2006 as a primary protein component of intracellular inclusions in most ALS cases, occurring in more than 90% of patients including those without mutations in TARDBP. This study has involved the development of antisense oligonucleotides (AOs) to modify expression of selected ALS-linked target genes, including TARDBP and FUS using an exon skipping strategy. Knockdown of RNA transcripts has been achieved using AOs synthesized as 2'-O-methyl modified bases on a phosphorothioate backbone. The evaluation of second generation clinically applicable morpholino oligomers is underway to evaluate effects on RNA and protein expression, cell survival and protein aggregation. The development of AOs that selectively target mutated alleles is also being investigated. AOs have shown great potential as therapeutics in treating neurodegenerative diseases but remain underexplored for many potential ALS targets. This work could lead to improved therapeutics for subsets of ALS patients. The AOs developed could also be utilized as tools in functional studies to help elucidate disease mechanisms.

G3

Gut Feelings about Parkinson's Disease: The Influence of the Gut Microbiota in a Rodent Model

Anastazja Gorecki[1,2], Leah Preskey[3,4], Megan Bakeberg[1,2], Jade Kenna[1,2], Christi Gildenhuys[1,4], Gabriella MacDougall[1,4], Sarah Dunlop[1,5], Frank L. Mastaglia[1,2], Anthony Akkari[1,2,], Frank Koengten[3], and Ryan S. Anderton[1,2,4*]

[1]Perron Institute for Neurological and Translational Science, [2]Centre for Neuromuscular and Neurological Disorders, UWA, [3]Ozgene Pty Ltd, , [4]Institute for Health Research, University of Notre Dame Australia, [5]School of Biological Sciences, UWA,

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in Australia, with motor, cognitive and gastrointestinal symptoms. Notably, gastrointestinal dysfunction can appear well before the onset of cardinal motor symptoms. Therefore, research has begun to focus on the interaction between the gut microbiota, gastrointestinal symptoms, and enteric alpha-synuclein (aSyn) aggregation in PD. The objective of this study was to investigate gut microbiota, and effects of an inflammatory lipopolysaccharide (LPS) trigger in a human αSyn over-expressing mouse model of PD (Thy1-αSyn). Stool samples from patients with confirmed PD and Thy1-αSyn mice were analyzed using 16S ribosomal RNA sequencing. Compared to healthy controls, the relative abundance of mucin-degrading Verrucomicrobiae and LPS-producing Gammaproteobacteria were greater in PD patients. In mice, the abundance of Gammaproteobacteria was negligible in both Thy1-αSyn and wild-type (WT) animals, while Verrucomicrobiae were reduced in Thy1-αSyn mice. The effect of LPS on intestinal barrier function was investigated *in vitro* using intestinal epithelial (IEC-6) cells, and *in vivo* via administration of LPS in drinking water to Thy1-αSyn mice. Acute exposure to LPS *in vitro* resulted in a reduction and altered distribution of the tight junction markers ZO-1 and e-Cadherin around the cell membrane in IEC-6 cells, as shown by immunohistochemistry. LPS administration in Thy1-αSyn mice resulted in the emergence of early motor manifestations at 10 weeks, compared to untreated mice who were still asymptomatic at this age. Overall, this study strengthens increasing evidence for a complex interaction between environmental and genetic determinants of PD, where dysbiosis and gastrointestinal dysfunction may act as a catalyst for αSyn pathophysiology and eventual neurodegeneration.

G4

Investigating the Guanidinium-Head Group of Poly-Arginine R18 Peptide: Implications for Cellular Uptake and Neuroprotection

Eden Ouliel[1,4], Gabriella MacDougall[1,4], Ryan S. Anderton[1,3,4] and Bruno P. Meloni[1-3]

[1]Perron Institute for Neurological and Translational Science, [2]Department of Neurosurgery, Sir Charles Gairdner Hospital, QEII Medical Centre, [3]Centre for Neuromuscular and Neurological Disorders, University of Western Australia, [4]School of Health Sciences and Institute for Health Research, University of Notre Dame Australia

Stroke is a leading cause of morbidity and mortality worldwide, therefore there is an urgent need for clinically effective neuroprotective agents to salvage vulnerable brain tissue after an ischaemic neurovascular event. Recent research has demonstrated that poly-arginine peptides have potent neuroprotective properties in *in vitro* and *in vivo* stroke models, with poly-arginine-18 (R18; 18-mer of arginine) peptide identified as a highly efficacious peptide. It has previously been established that the presence of arginine residues can enhance peptide uptake and neuroprotection, and it is hypothesized that this occurs by way of the guanidinium head group, a structure found exclusively on arginine. Therefore, this study aimed to investigate the role of the guanidinium head groups in R18 cellular uptake, intracellular localisation and neuroprotective function. Comparative studies were performed using R18 and poly-ornithine-18 (O18; 18-mer of ornithine). O18 displays the same net positive charge as R18 and is structurally identical to R18, but does not possess guanidinium head groups. Cellular uptake and localisation of FITC-conjugated R18 and O18 peptides was examined in SH-SY5Y neuroblastoma cell and primary cortical neuronal cell cultures using confocal microscopy. R18 and O18 were also assessed for neuroprotection in a neuronal glutamic acid excitotoxicity model, with fluorometric and colourmetric assays performed to examine neuronal calcium influx, calcium-mediated caspase and calpain activity and cellular viability. Both peptides demonstrated uptake in both cell types, with localisation differing in neurons. In the excitotoxicity model, R18 was highly neuroprotective and significantly reduced intracellular calcium influx, whereas O18 displayed no neuroprotection, and a nonsignificant effect in reducing calcium influx. Fluorometric assays revealed R18 attenuated calcium-mediated caspase-3, 7 and 9 and calpain activation at 6- and 24-hours post-injury, whereas O18 was ineffective. Overall this study indicates that the guanidinium head group does not appear to induce peptide uptake, but is a critical factor for neuroprotection.

G5

Early Neurodegeneration in the Peripheral Nerves of the *Dmd*^{mdx} Rat Model for Duchenne Muscular Dystrophy

Lakshana Parameswari Thanigaiarasu[1], Vidya S Krishnan[1], Miranda D Grounds[1]

[1]School of Human Sciences, The University of Western Australia

Introduction. The dystrophic *Dmd*^{mdx} rat is a new model for Duchenne muscular dystrophy (DMD) that displays a more severe phenotype than the *mdx* mouse. In *mdx* mice, neurodegeneration (resulting from repeated myonecrosis) is observed in sciatic nerves by 13 months of age. This study tests whether such neurodegeneration occurred earlier, at 8 months, in the *Dmd*^{mdx} rat model. **Problem Statement**. To identify neurodegenerative changes, immunoblotting was used to measure levels of various proteins associated with peripheral nerve function, including Tau5 and S100, SMI-32, P62, gelsolin and vimentin, for sciatic and radial nerves of 8-month-old *Dmd*^{mdx} and control wild-type (WT) rats. **Procedure**. Total protein was extracted from the nerves (n=8/group), protein concentration measured, and proteins of interest were immunoblotted and quantified by densitometry analyses, normalised to total protein levels. Data from the sciatic and radial nerves were compared separately using unpaired student's t-test. **Results**. Tau5 protein levels were ~30-40% higher in *Dmd*^{mdx} sciatic nerves, compared with WT ($p<0.001$). Similarly, S100 levels in *Dmd*^{mdx} sciatic nerves were ~40% higher than for WT ($p<0.05$). However, for radial nerves, there were no significant differences between the 2 strains in any of the proteins. **Conclusions**. Increased levels of Tau5 and S100 proteins in dystrophic sciatic nerves but not in radial nerves indicate early neurodegeneration in these nerves of 8-month *Dmd*^{mdx} rats. This premature neurodegeneration (probably irreversible) indicates ongoing necrosis in dystrophic rat muscles and further validates the severity of the new *Dmd*^{mdx} rat model. This neurodegeneration is likely to be progressive and contribute to loss of function in dystrophic muscles, especially after many years in young DMD boys. Such protein markers of early neurodegeneration could provide as useful new readouts for preclinical studies to measure long term benefits of therapies, specifically aimed to protect dystrophic muscles from myonecrosis in DMD.

G6

Is a Decline in Neurocognitive Function the Consequence of a High Burden of CMV?

Phuongnhi Ha[1], Silvia Lee[1], Michael Vacher[2], Simon Laws[3], Patricia Price[1]

[1] Curtin Health Innovation Research Institute, Curtin University, Bentley, Australia, [2] Commonwealth Scientific and Industrial Research Organisation, Perth, Australia, [3]School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia

Human Cytomegalovirus (CMV) is a beta-herpesvirus that has evolved a complex and ancient relationship with humans. CMV establishes lifelong latent infections in its host and has a seroprevalence of 30-100% worldwide depending on factors such as age, location and socioeconomic status. Infection with CMV can be severe in immunocompromised people and has been associated with increased mortality risk and poorer health in the elderly. Reactivation of CMV can lead to clinical manifestations such as vasculopathy and organ transplant failure. We sought to investigate the effect of CMV on the development of Alzheimer's disease in the elderly by using antibodies as a measure of the burden of CMV in an individual. In a subset (n=200) of Caucasians in the Australian Imaging Biomarkers Lifestyle study of Aging (AIBL), IgG antibodies reactive against a lysate of CMV-infected fibroblasts, recombinant CMV glycoprotein B (gB) or CMV Immediate-Early protein 1 (IE-1) were quantitated using an in-house indirect ELISA. Antibodies were quantitated against a standard assigned a value in arbitrary units. Using bivariate analysis, we observed a weak positive correlation of CMV-reactive antibodies on each cognitive domain (ie. episodic memory, recognition, executive function, language and attention). Anti-gB and anti-CMV-lysate antibodies had the clearest correlation on executive function reflecting control, working memory and cognitive flexibility ($p=0.05$ and $p=0.07$, respectively). Additionally, individuals with amyloid beta deposits detectable by PET had lower levels of CMV-reactive antibodies detected with IE-1 ($p=0.02$) or CMV-lysate ($p=0.04$). It may be critical that CMV-reactive antibody levels did not rise with age in this population (59-89 years old). We postulate that CMV-reactive antibodies may be protective of neurocognitive function in the elderly. Whilst this appears to conflict with links between CMV seropositivity per se and Alzheimer's disease, it is possible the CMV-reactive antibodies control viral replication in older Caucasian Australians.

G7

Antisense Oligonucleotide-Mediated Exon Skipping to Treat Spinocerebellar Ataxia Type 3

Craig S McIntosh[1,2], May Thander Aung-Htut[1,2], Sue Fletcher[1,2] and Steve D Wilton[1,2]

[1]Molecular Therapy Laboratory, Centre for Molecular Medicine and Innovative Therapeutics [2]Perron Institute for Neurological and Translational Science

Introduction.Spinocerebellar ataxia type 3 (SCA3) is a devastating neurodegenerative disease, which is one of nine polyglutamine disorders. Although SCA3 is pathogenically heterogeneous, the main feature is progressive ataxia, which in turn affects speech, balance and gait of the affected individual. There is currently no cure, nor effective treatment strategy for affected individuals. SCA3 is caused by an expanded polyglutamine tract found in ataxin-3, resulting in conformational changes that lead to toxic gain of function. This expanded glutamine tract is located at the 5' end of the penultimate exon (exon 10) of *ATXN3*. **Problem Statement**. This study aims to use antisense oligonucleotide (AO) mediated exon skipping to develop a therapeutic strategy for the treatment of SCA3. **Procedures**. AOs were designed to target sequences critical to pre-mRNA processing in an attempt to disrupt splicing and induce skipping of the targeted exon (Exon 10). SCA3 patient cells were transfected with AOs for 48 hours. Cells were lysed, and subsequent RT-PCR and western blot analyses were conducted to determine the effects of the transfection on the mRNA transcript and protein, respectively. **Results**. Initial *in vitro* data using 2'-O-methyl AOs in patient cells show that it is possible to create an internally truncated protein, missing the toxic CAG repeat and still maintain normal function of the protein. Confirmatory data using the clinically relevant phosphorodiamidate morpholino oligomer (PMO) chemistry showed complementary positive results to 2'O-methyl data. Additionally, significant downregulation of both the mutant and wild-type protein was observed, allowing for a combination of benefits. However, PMO is considered to be a superior chemistry when compared to 2'-O-methyl, as they are chemically stable and have an excellent safety profile to date. Further data shows that PMO chemistry is longer lasting and significantly better tolerated by cells. **Conclusions**. This study provides a possible therapeutic strategy to treat SCA3.

G8

Antisense Oligomer-induced Exon Skipping to Restore Dysferlin Function in LGMD 2B Patients

Bal Hari Poudel[1,2,3], Dr Loren Flynn[1,3], Prof Sue Fletcher[1,3], Prof Rohit Pokhrel[2] , Prof Norman Palmer[1,3] and Prof Steve Wilton[1,3]

[1] Center for Molecular Medicine and Innovative Therapeutics, [2] Tribhuvan University, Nepal, [3] Perron Institute for Neurological and Translational Science

Dysferlin (*DYSF*) is a calcium-dependent membrane-associated protein implicated in membrane repair, vesicle trafficking, and T-tubule function. Mutation in *DYSF,* encoding the dysferlin protein cause primary dysferlinopathies; a group of autosomal recessive diseases that includes Limb-Girdle Muscular Dystrophy type 2B (LGMD 2B), Miyoshi Myopathy and Distal Myopathy. More than 350 pathogenic mutations in *DYSF* has been reported within exons or near an intron-exon boundary. Due to the large size of the *DYSF* mRNA, AAV-mediated gene transfer is challenging because the protein coding sequence exceeds the capacity of a single AAV vector. We hypothesize that antisense-mediated exon skipping strategies could be applicable to particular dysferlin mutations and restore dysferlin function. Antisense oligomers (2´*O*-methyl modified bases on a phosphorothioate backbone were designed to skip exons 2, 3, 4, 25, 30, 32, 34, 35, 36, 37, 51 and 52 transfected into healthy human myoblasts. The antisense oligomers targeting exons 30 and 32 induce almost 100% skipping at a transfection concentration of 400 nM. The most effective exon 32 targeting sequence was synthesized as phosphorodiamidate morpholino oligomer, evaluated for exon 32 skipping in patients myogenic cells and showed 100% exon 32 skipping by RT PCR analysis, verified by sequencing. Further analyses of treated cells by western blotting and functional assays are currently underway. The Loss of *DYSF* exon 32 had been associated with a milder presentation of LGMD 2B, and antisense mediated skipping of additional exons will be evaluated. Thus, exon skipping appears to be a viable strategy to overcome *DYSF* mutations affecting exon 32 and we will explore the redundancy of other exons.

Australian Herding Dog Breeds Pawsitive for New Disease Mutations

Jade Waters[1]

School of Pharmacy and Biomedical Science Curtin University[1], Curtin Health Innovation Research Institute[2]

Dogs, like humans, are affected by diseases with Mendelian inheritance. These diseases are typically associated with particular breeds but may have wider distribution and genetic testing is not commonly undertaken. A recent study, using dogs mostly from the US and Europe, found that many breeds carry mutations for genetic diseases with which they hadn't previously been associated. In Australia herding breeds, such as the Border Collie, Kelpie, Australian Shepherd, Koolie, Australian Cattle Dog and Stumpy Tailed Cattle dog, are commonly kept as companions and/or valuable working dogs. It is important to determine if the Australian population of these breeds are also associated with these new diseases. Focus was placed on two recessive Mendelian diseases, Collie Eye Anomaly (CEA) and von Willebrand disease type 2 (VWD type 2). A fragment length polymorphism polymerase chain reaction (RFLP-PCR) was designed to detect the 7.8kb deletion that causes CEA, whilst a tetra-primer amplification refractory mutation system polymerase chain reaction (Tetra-primer ARMS) was used to detect the single nucleotide polymorphism associated with VWD type 2. Both these methods can accurately identify affected, unaffected and carrier individuals for the relevant disease. It was calculated that 101 samples from each breed would be needed to determine the significance of the findings. The results identified the mutation for CEA in Kelpies, in its carrier form, and the VWD type 2 mutation was identified in Border Collies, also in its carrier form. However, the clinical significance of these findings cannot yet be determined, because the study is ongoing. To this point, this study has not identified any genetically affected individuals in breeds that have not previously been associated with CEA or VWD type 2. If such an affected individual is identified, phenotype testing will be needed to confirm if these mutations result in the expected clinical syndrome.

Splice Modulating Antisense Oligonucleotides to Address COL3A1 Mutations Causing Vascular Ehler Danlos Syndrome

Sasiwimon Utama[1,2], Chalermchai Mitrpant[1], May T. Aung-Htut[2,3], Sue Fletcher[2,3], Steve Wilton[2,3]

[1]Faculty of Medicine Siriraj hospital, Mahidol University, Thailand, [2] Center for Molecular Medicine and Innovative Therapeutics, Murdoch University [3]Perron Institute for Neurological and Translational Science

Introduction. Vascular Ehler Danlos syndrome or Ehler Danlos syndrome type IV (vEDS or EDS type IV) is a connective tissue disorder characterized by skin hyperextensibility, joint hypermobility and fatal vascular rupture. vEDS is caused by *COL3A1* mutations that disrupt collagen III expression. COL3A1 is classified as fibrillar collagen, along with collagens I, II, V and XI in the extracellular matrix, particularly in the inner organs such as uterus, bowel, blood vessels, etc. To date, only symptomatic treatment for vEDS patients is available. **Problem statement.** vEDS patient fibroblasts carrying an IVS14-2G>A mutation in COL3A1 show exon 15 skipping and reduced COL3A1 secretion compared to normal fibroblasts. In consistent with patient fibroblasts carrying a single base deletion (c.766delA) in exon 10, have the reading-frame disrupted and there was a reduction of secreted collagen III, presumably due to compromised COL3A1 homo-trimer fibril assembly. The aim of this study is to increase collagen III secretion in these patient fibroblasts using splice switching antisense oligonucleotides (AOs). We hypothesize that removal of the mutated exon from both the normal and disease causing *COL3A1* alleles would by-pass the mutation and produce COL3A1 isoforms capable of trimerization, thereby increasing collagen III secretion. **Procedures and Results.** Screening of three 2'O-methyl phosphorothioate AOs (2OMeAO) to skip exon 10 in patient fibroblasts did not show detectable exon 10 skipping when applied individually, but the combination of AO2 and AO3 induced 40% skipping of the targeted exon. These two AO sequences were resynthesized as phosphorodiamidate morpholino oligomers (PMOs) and induced 100% exon 10 skipping in both patient and normal fibroblasts. COL3A1 exon 15 was efficiently excised from the mature mRNA with 2OMeAOs and with the equivalent PMOs. Western blot was used to assess the collagen III secretion and immunofluorescence staining identified collagen III deposition in the extracellular matrix.

Characterizing Stargardt Disease-causing Mutations to Identify Gene Lesions Amenable to Splice Intervention Therapies

Di Huang[1], Sue Fletcher[1], May Thandar Aung-Htut[1], Jennifer A. Thompson[3], John N. De Roach[2,3], Samuel McLenachan[2,4], Shang-Chih Chen[4], Fred Chen[2,3,4], Steve Wilton[1]

[1]Centre for Molecular Medicine and Innovative Therapeutics & Perron Institute, [2]Centre for Ophthalmology and Visual Science, [3]Australian Inherited Retinal Disease Registry and DNA Bank, [4]Lions Eye Institute

Introduction. Stargardt disease (STGD1) is an autosomal recessive juvenile-onset macular dystrophy, characterized by bilateral progressive impairment of central vision and subretinal extra-cellular and intra-cellular deposition of lipofuscin-like substances in the macula and/or midperipheral retina. STGD1 is caused by mutations in the ATP-binding cassette transporter gene (*ABCA4*) that encodes a transmembrane protein responsible for transportation of all-*trans*-retinal-conjugated molecules, sourced from both photobleached rhodopsin and cone opsins in the photoreceptor outer segments (OS), and chromophore contained within the visual pigments of phagocytosed OS discs in retinal pigment epithelial cells. **Problem Statement**. Although biallelic *ABCA4* variants are identified by DNA analysis in approximately 60~70% of STGD1 patients, the consequences on splicing of the transcript and gene expression remain unknown. **Procedures.** We recruited a cohort of 65 STGD1 patients who had pathogenic, or likely pathogenic, *ABCA4* variants. Patient-derived fibroblasts were cultured from skin biopsies and the *ABCA4* transcripts were analyzed using RT-PCR to determine consequences of the genetic change on *ABCA4* transcript structure. **Results**. Interestingly, we were unable to amplify exons 1-12 of the *ABCA4* transcript from fibroblast RNA, whereas the remainder of the transcript (exons 13-50) of this retinal-specific gene could be amplified. Six of the *ABCA4* variants were found to affect pre-mRNA processing: (*ABCA4* c.5461-10T>C, c.4773+3A>G, c.5835+1G>A, c.4919G>A, c.6031_6044delinsAGTATTTAACCAATATTT and c.5197-4C>A), generating aberrantly spliced *ABCA4* isoforms. **Conclusions**. Patient-derived fibroblasts provide a valuable resource for identifying splicing defects potentially amenable to antisense oligonucleotide-mediated splice intervention therapies.

Association of a Poly-T Structural Variant within the *SCAF4* gene and Amyotrophic Lateral Sclerosis

Julia Pytte[1,2], Loren L Flynn[2,3], Frances Theunissen[1,2], Ryan S Anderton[2,4], Ian James[3], Ann M Saunders[5], Richard Bedlack[5], Teepu Siddique[6], Ornit Chiba-Falek[5], Michael Lutz[5], Allen D Roses[5], Anthony Akkari[2,3]

[1]Human Sciences, University of Western Australia, [2]Perron Institute [3]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, [4]Health Sciences, University of Notre Dame, [5]Department of Neurology, Duke University and Durham VA Medical Centre, Durham, NC, USA, [6]Department of Neurology, The Les Turner ALS Research & Patient Centre, Northwestern University Feinberg School of Medicine, Chicago

Introduction. There are currently over 200 reported fALS associated genetic variants in the *SOD1* gene. The functional significance of these associated variants remains largely undetermined. Structural variants (SVs) are highly polymorphic markers that have been implicated in altered gene function and can potentially explain significant phenotypic variability observed in complex disorders, such as ALS. Consequently, we have examined SVs within and surrounding the *SOD1* gene loci to assess their relevance to ALS. **Problem Statement.** SVs within and around the *SOD1* gene may be associated with disease and phenotypic variation in ALS cohorts. **Procedures.** A SV evaluation algorithm was used to identify and score candidate variants according to variability, proximity to regulatory elements, trait association, signal for transcription factor binding sites, conservation and intron size. A selected variant termed SV1 was systematically assessed through polymerase chain reaction, capillary separation and Sanger sequencing. **Results.** In the present study SV1, a 15-18 Poly T repeat, was identified in *SCAF4*, a gene downstream of *SOD1*. In a North American cohort of familial (n=180) and sporadic (n=29) ALS patients and age-matched healthy controls (n=555), we subsequently showed that carrying an 18T SV1 allele is associated with ALS (P=0.001). Furthermore, carriage of an 18T allele was strongly associated with a shorter survival by an average of 33 months (P=0.001). **Conclusions.** The potential association between SV1 length, *SOD1* variants and fALS was examined in this study. From a biomarker perspective, common *SOD1* ALS associated variants are not incredibly informative for disease. In this study we present a highly informative biomarker marker for ALS risk and survival. This is the first report of such an SV in the *SCAF4* gene and highlights the importance and implications of further investigation into SVs that may provide new targets for cohort stratification and therapeutic development.

Making the Inactive Active through Changes in Antisense Oligomer Chemistries

Khine Zaw[1,2,3], Kane Greer[1,2], May Thandar Aung- Htut[1,2], Rakesh Veedu[1,2], Chalermchai Mitrpant[2,3], Sue Fletcher[1,2], Steve Wilton[1,2]

[1]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, [2]Perron Institute for Neurological and Translational Science, Western Australia, [3]Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Antisense oligomers (AOs) are short, single-stranded nucleic acid analogues that may anneal to a mRNA or pre-mRNA by complementary base pairing and, depending on the base and backbone chemistries, can induce a variety of mechanisms to alter the gene expression. We have designed AOs to redirect the splicing process and have used this strategy to by-pass disease-causing mutations in the dystrophin gene (*DMD*) by inducing a specific exon-skipping to restore the reading frame. When developing a panel of AOs to skip all dystrophin exons, remarkably we found that two out of three compounds could induce some exon skipping, while one in three AOs appeared to be completely ineffective. In this study, we modified the composition of some ineffective AOs and increased their annealing potential by incorporating locked nucleic acid residues into the sequences. AOs targeting the skipping of exon 16, 51 and 23 of human *DMD* transcripts were synthesized as two different chemistries, 2'-O-methyl (2'-OMe) RNA-like oligos and locked nucleic acid (LNA)/2'-OMe mixmers. Primary human myoblasts were treated with 2'-OMe or LNA/2'-OMe AOs, and the *DMD* transcripts were analysed by for exon skipping. As expected, the ineffective 2'-OMe AOs showed no exon skipping while all the LNA/2'-OMe mixmers exhibited skipping of the targeted exons. Interestingly, the LNA/2'-OMe mixmer targeting exon 51 induced two additional transcripts, representing incomplete skipping of exon 51 with retention of 95 or 188 bases from the beginning of exon 51. These results indicate that LNA/2'-OMe mixmers may be more efficient at exon skipping, but this improvement may come at the cost of activating alternate cryptic splice sites.

Utilisation of FISH as a Diagnostic Adjunct for Pancreatobiliary Tract Malignancy on Cytology Cell Block Specimens

Shay Zimmerle[1], Dr Ben Dessauvagie[2], Dr Alina Miranda[3] , Dr Chris Van Vliet[4], Ms Joanne Peverall[5]

[1]Curtin University, [2]PathWest, [3]University of Western Australia

Malignancies of the pancreatobiliary (PB) tract are notoriously aggressive and associated with dismal prognoses. Establishing an early and accurate diagnosis is essential to ensure the best possible outcomes for patients. Clear-cut diagnoses can be challenging to achieve due to a non-specific clinical presentation, radiological findings that overlap with inflammatory conditions and tumour cells on small biopsy, and/or cytopathology specimens with subtle features of malignancy. These variables can delay a definitive diagnosis and subsequent treatment. A fluorescence in situ hybridisation (FISH) probe panel has been identified that improves clinical sensitivity up to 93%; however, cytology sampling to carry out the test required proprietary equipment associated with time and cost implications that would hamper clinical uptake. It is our hypothesis that the test can be mimicked utilising a set of locus-specific indicator probes designed to target MCL1, EGFR, c-MYC, and CDKN2A on routinely collected cytology samples without the need for specialised equipment. We have successfully validated this set of probes on formalin fixed and paraffin embedded PB histopathology specimens from 48 samples known to be normal or abnormal, and cut-off values for normal and abnormal samples were derived from scorings of at least 50 nuclei from each hybridisation. This test has the potential to be transformative for PB tract malignancy as it can be carried out on routinely collected and handled samples without the need for specialised techniques, training, or equipment, resulting in a higher proportion of clear-cut benign or malignant diagnoses, and a lower risk of delay for potentially life-saving treatment.

H7

Variation in Promoter Sequences Drives Differential Expression of GLORF-C4 in the Human Infective Assemblages of *Giardia duodenalis*

Amy Black[1,2], Dr. Carl Mousley[1,2], Professor Tim Paget[3], Dr. Robert Steuart[2]

[1]School of Biomedical Science, [2]Curtin Health Innovation Research Institute, Curtin University, Perth, Australia; [3]Pharmacy, Pharmaceutical and Cosmetic Sciences, University of Sunderland

Giardia duodenalis, an important human pathogen, has a two-stage lifecycle; the cyst and trophozoite. There are eight distinct genetic groups, two of which, assemblages A and B infect humans. Encystation is the encasement of the trophozoite by cyst wall material and is a major virulence determinant. Previous proteomic work from our lab has found GLORF-C4 protein exclusively in assemblage B trophozoites. Sequential work identified low levels of *glorf-c4* mRNA in assemblage A trophozoites. Therefore, we wished to determine the differential expression of GLORF-C4 and the basis for this expression. We concluded that GLORF-C4 in assemblage B isolates is constitutively expressed whereas expression is significantly increased upon the induction of encystation in assemblage A cells. Examination of the upstream sequence of both the assemblage A and B GLORF-C4 genomic sequences identified a 14bp INDEL. In assemblage B this region is homologous to known prokaryotic promoter motifs, which is disrupted in assemblage A. To determine whether there is any difference in encystation rate between the human infective forms, potentially due to the INDEL, we induced assemblage A and B isolates to encyst and sampled cells every 6 hours for 72 hours. We found there was a similar trend in cyst wall protein positive cells (encysting cells and cysts) throughout the encystation time course between assemblages A and B. However, assemblage B cells were able to convert encysting cells to cysts faster than assemblage A. GLORF-C4 has structural homology to heat shock protein 26 (HSP26). HSP26 is induced during stress conditions in other organisms however, no obvious HSP26 has been identified in *Giardia*. Encystation can be deemed a stress response. Therefore, GLORF-C4 has a potential role as a HSP26 in *Giardia* during encystation. Additionally, the constitutive expression of GLORF-C4 in assemblage B cells may be beneficial for cyst production during encystation.

H8

Impact of Viral Adaptation During Early HIV Infection on Disease Outcome

Alves E[1], Keane N[2], John M[2,3], Chopra A[2,4], Leary S[2], Almeida C[2], Currenti J[1], Deshpande P[1], Smith R[4], Mallal S[2,4], Kalams S[4], Gaudieri S[1,2,4]

[1]Human Sciences, UWA, WA, Australia, [2]Institute for Immunology and Infectious Diseases, Murdoch University, Australia, [3]Department of Clinical Immunology, Royal Perth Hospital, Australia, [4]Infectious Diseases, Department of Medicine, Vanderbilt University, USA

Human immunodeficiency virus (HIV) remains a worldwide health risk, having claimed over 35 million lives to date. No cure for HIV exists, partially due to the virus' high mutation and replication rate. Due to these characteristics, HIV has been shown to adapt and consequently evade an individual's immune response. Unfortunately, current HIV vaccine design approaches do not sufficiently incorporate these viral adaptations, and it has become increasingly apparent that these must be considered for an effective vaccine. Previous research has explored the detrimental impact of these adaptations in the late stage of HIV infection, however more research is needed into the impact of these adaptations in early infection – especially since changes during this period strongly influence disease progression. Our study analysed the composition of the three most commonly considered HIV viral proteins in vaccine design - *Gag, Pol* and *Nef* - using next-generation sequencing across the early stage of infection from 10 individuals, in order to explore when these adaptations arise and what impact they have on the individual's disease status. We show that these adaptations appear early on, increase in number over time, and appear to be associated with the amount of virus in the individual's blood. Pinpointing and understanding these early adaptations has become fundamental as individuals are being placed on treatment as soon as infection is suspected. Our research will aid current vaccine design prospects for HIV by improving the understanding of the extent to which the virus can adapt in these early stages, which regions of the virus readily adapt, and how these influence the host's survival.

H9

Global HCV Adaptation to Host Immunity Polymorphisms

Yueran Li[1], Pooja Deshpende[1,2], Silvana Gaudieri[1,2,3].

[1]School of Human Sciences, University of Western Australia, [2] Institute of Immunology and Infectious Diseases, [3] Vanderbilt University

Hepatitis C infection is estimated to affect 2.6-3% of the world population, which might progress to Cirrhosis and Hepatocellular carcinoma. HCV is highly diverse and has high mutation rate. The current treatment does not prevent reinfection with different strains. Understanding host and pathogen interaction is crucial for future vaccine design. Human Leukocyte Antigen (HLA) Class I molecules transport and present mature viral epitopes to the surface of infected cells, for the recognition by CD8+ T cell and NK cell receptors, which induce immune responses. HLA polymorphisms are associated with HCV adaptations. The precursor viral peptides undergo further cleavage by Endoplasmic Reticulum Aminopeptidase (ERAP) to the optimal length (8-10 mer) for binding to the HLA Class I molecules. The Single Nucleotide Polymorphisms (SNPs) in ERAP results in different peptide trimming efficiencies hence might affect HLA antigen binding. It is hypothesized that immunodominant HLAs are associated with great proportion of HCV adaptations; also, hypoactive ERAP allotypes are associated with reduced HCV adaptation. Patients' blood samples (n=210) were obtained to isolate viral RNA and host DNA. The Viral RNA sequencing and host HLA typing were previously performed using Sanger-based sequencing method. ERAP genotyping was performed using Next Generation Sequencing on Illumina Platform. For each individual, numbers of Amino Acid (AA) changes from consensus of the studied cohort were recorded as overall adaptations. Numbers of AA changes at published HLA-restricted adaptation sites were recorded to investigate HLAs' contribution to HCV adaptation. The data had shown that most of the HCV adaptations were at the HLA restricted adaptation sites; individuals with hypoactive ERAP phenotypes showed reduced overall adaptations. Immunodominant HLA genotypes have great contribution to HCV adaptations; however, the presence of hypoactive ERAP phenotypes could counteract this effect.

H10

Genetic Exchange in Giardia Duodenalis

Lamprini Baklous[1,2], Carl J. Mousley[1, 2], Rob F. L. Steuart[2]

[1]School of Pharmacy and Biomedical Sciences, [2]Curtin Health Innovation Research Institute, Curtin University, Perth, WA

Introduction. *Giardia* species are recognized as one of the earliest diverging eukaryotes. *Giardia duodenalis* is the most commonly reported intestinal parasites of humans, affecting around 280 million people worldwide. Assemblages A and B are the two genetic groups known to infect humans. It is unknown if *Giardia* species genomes can recombine with each other. However, genetic comparisons of human-infecting *Giardia* Assemblages A and B suggest that genetic exchange does occur. Opportunities for genetic exchange in the giardia lifecycle may coincide with expression of meiosis-associated recombination machinery, however, using current technology it is difficult to identify if and when these genes are expressed. Several genes present in the *G. duodenalis* genome are known to exhibit meiosis-specific expression in higher eukaryotes. **Problem Statement**. We hypothesise that *Giardia duodenalis* is capable of exchanging nuclear genetic material between different assemblage A and B populations. **Procedures.** We will monitor expression of meiosis-specific genes throughout the giardia lifecycle using fluorescent protein reporters and fluorescence microscopy to facilitate visual detection of recombination-gene expression. For comparison, the same genes will be analysed in assemblages known to have less evidence for gene exchange. Mixtures of assemblages modified to carry plasmids constitutively expressing fluorophores will then be mixed and observed for evidence of plasmid transfer. **Results**. We have successfully designed and optimised PCR reactions for 11 of the core meiotic genes. Initial results indicate varied expression between the different genetic groups at similar time points in the encystation process. **Conclusions**. The results so far have provided evidence for the presence of meiosis-specific genes and their expression during the process of encystation in both assemblages A and B. This study may provide information on how anti-microbial resistance could be transferred between isolates. The confirmation of meiotic recombination within the *Giardia* species will provide the firsts steps to finding the origins of meiotic genetic recombination making it an important part of evolutionary biology.

Role of Oxidoreductases and Phosphoethanolamine Lipid A Transferase of *Neisseria gonorrhoeae* on Survival in Macrophages

Ethan C. Haese[1], Katherine Y. L. Lim[1,2], Charlene M. Kahler[1]

[1]Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Western Australia, [2]School of Molecular Sciences, Faculty of Science, University of Western Australia

If the current trend of increasing antimicrobial resistance continues, by 2050 around 10 million people may die every year as a direct result of untreatable infections. In the past two decades, multi-drug resistant gonococcal (MDR-GC) strains with decreased susceptibility to extended-spectrum cephalosporins, such as cefixime and ceftriaxone, have spread around the world, resulting in treatment failures. The World Health Organisation has recommended that research focus on identifying novel therapies for treatment of MDR-GC strains. The enzymes involved in protein folding pathways and lipooligosaccharide (LOS) modification are excellent targets for novel anti-virulence strategies. The neisserial phosphoethanolamine lipid A transferase enzyme EptA provides protection against non-oxidative antimicrobial factors produced by neutrophils and macrophages, including cationic antimicrobial peptides (CAMPs). Lipid A moieties of LOS are decorated with phosphoethanolamine by EptA before being exported to the outer leaflet of the outer membrane, providing resistance to the bactericidal activity of CAMPs. EptA is an inner membrane bound protein that contains five disulphide bonds in its periplasmic soluble domain, indicating that it is a substrate of the oxidative protein folding pathways in the bacterial periplasm. *N. gonorrhoeae* contains two oxidoreductases, DsbA1 and DsbA3. Both oxidoreductases have the capacity to introduce disulphide bonds into EptA, however, only the loss of DsbA3 has been shown to result in protein instability and loss of function in *N. meningitidis*. The hypothesis of the project is that the oxidoreductase pathways are required for EptA stability and are essential to the survival of *N. gonorrhoeae* in macrophages. The aims of this study are then as follows: (1) to construct EptA, DsbA1, and DsbA3 knockout mutants in *N. gonorrhoeae* and test for EptA activity; (2) to characterise the mutants for the expression of downstream outer membrane virulence factors; and (3) to test the survival of the mutants in a murine macrophage model.

Investigations into Transient Antibiotic Resistance in Bacteria Exposed to Antimicrobial Peptides.

Karan R. Bamb[1,2], Joshua P. Ramsay[1,2], Ranil Coorey[1,3], Gary A. Dykes[1,4], Joshua T. Ravensdale[1,4]

[1]Curtin Health Innovation Research Institute, Curtin University, School of Pharmacy and Biomedical sciences, Curtin University, [3]School of Molecular and Life sciences, Curtin University, [4]School of Public Health, Curtin University, Perth Western Australia

Antibiotic resistance in bacteria is one of the most important public health concerns globally. Infections caused by these bacteria are difficult to treat with conventional antibiotics. Therefore, new therapeutic options with different modes of action to antibiotics and low toxicity to host cells are required. Antimicrobial peptides (AMP) are short and hydrophobic sequences of amino acids that are can be toxic to a broad range of microorganisms. Cationic peptides act by binding to anionic regions on the bacterial membrane and disrupting the phospholipid bilayer by inserting themselves into the hydrophobic core of the membrane. Numerous studies have shown bacteria are able to develop resistance through prolonged exposure to the peptides. Resistance to the peptides may cause a substantial alteration to the fundamental physiology of the cell which could alter the sensitivity of the bacteria to other antimicrobial agents and lead to transient antibiotic sensitivity or resistance. This project will investigate the changes in the antibiotic sensitivity profiles in multi-drug resistant (MDR) *Staphylococcus aureus* strains which have been exposed to increasing concentration of AMPs over seven days. The antibiotic sensitivity profile for these strains will be tested against the 6 main classes of antibiotics over the seven day subculture. If a change in the antibiotic sensitivity profile of these *S.aureus* strains is observed, RNAseq will be performed on these strains following exposure to the peptides to compare the transcription profiles of AMP-resistant *S.aureus* strains. RT-qPCR will be carried out to confirm the changes in the transcriptional profile. The expression profile of known antibiotic resistance genes will also be monitored to examine if these genes can contribute towards resistance to the peptides. Data from this study could help identify the potential for transient resistance towards antimicrobial agents in *S. aureus* which could lead to new strategies to combat antibiotic resistance in bacteria.

I3

Characterisation of Mips and EptA as anti-virulence targets in *N. meningitidis*

Anubhav Khandelwal[1], Emily A. Kibble[1], Mitali Sarkar-Tyson[1] and Charlene M. Kahler[1]

[1]Marshall Centre for Infectious Disease Research and Training, School of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Western Australia

The meningococcal human pathogen, *N. meningitidis*, represents a global public health concern with its increasing resistance to first-in-line antibiotics, penicillin and ceftriaxone, and restricted inability for alternative antibiotics to cross the blood brain barrier. There is a pressing need to utilise innovative therapeutic strategies to overcome high resistance, while one strategy is to develop drugs that inhibit virulence determinants, thus improving the ability of the human immune system to clear the infection. Two novel virulence-associated proteins in *N. meningtidis* are the macrophage infectivity potentiators (Mips), which exhibit peptidyl-prolyl *cis/trans* isomerase activity involved in protein folding pathway, and the phosphoethanolamine transferase (EptA) enzyme responsible for lipooligosaccharide (LOS) lipid A modification. Previous data showed that Mips and EptA are separately required for survival in macrophages. Here, the combined effects of targeting both Mips and EptA on macrophage survival will be examined. To investigate this, triple and double mutants, Δ*mip1*Δ*eptA*, Δ*mip2*Δ*eptA* and Δ*mip1*Δ*mip2*Δ*eptA* were constructed by natural transformation and analysed. There was no growth defect observed amongst the mutant strains compared to wild type NMB. To determine the sensitivity to cationic antimicrobial peptides, the mutants were tested for susceptibility to polymyxin B (PxB) using broth microdilution method and later confirmed by Epsilometer test (E-test). The Δ*mip1*Δ*mip2*Δ*eptA* mutant presented with a significant reduced ability to counteract the bactericidal activity of PxB (MIC ≤ 0.125 μg/mL). This result was identical to the MICs of double mutants Δ*mip1*Δ*eptA* and Δ*mip2*Δ*eptA*, along with the single Δ*eptA* mutant. The ability of these *N. meningitidis* mutant strains to survive macrophage killing mechanisms is being investigated *in vitro*. These results will determine if targeting Mips and EptA in combination will be an effective strategy to ultimately disarm and deliver efficient clearance of meningococci by the host immune system.

I4

Exploring the Therapeutic Potential of Phage Therapy to Treat Pseudomonas Aeruginosa Infection in People with Cystic Fibrosis

Renee N Ng[1,2], Andrew Vaitekenas[3], Matthew W.P. Poh[2], Daniel R. Laucirica[2], Scott Winslow[2], Jessica Hillas[2], Stephanie Trend[2,4], Barbara J. Chang[5], Stephen M. Stick[2,4,6], Anthony Kicic[2,4,6,7] on behalf of WAERP and AREST CF

[1]Biomedical Sciences, UWA, [2]Telethon Kids Institute, [3]Pharmacy and Biomedical Sciences, Curtin University., [4]Division of Paediatrics, UWA, [5]The Marshall Centre for Infectious Diseases Research and Training, [6]Dept of Respiratory Medicine, PCH, [7]School of Public Health, Curtin University

Antimicrobial resistance is a global health crisis, which has accelerated due to overuse of antibiotics as microorganisms develop resistance when exposed to antimicrobials. There is an urgent need to develop new therapeutics and we propose the use of bacteriophages, or "phages" as an alternative to antibiotics. Multidrug resistance in microorganisms affects people with accompanying illnesses such as Cystic Fibrosis (CF) more severely. CF, an inherited genetic disease caused by mutations in the CFTR gene, leads to mucus build-up in the lungs of patients, creating an ideal environment for the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*). Most CF patients will be persistently colonised by *P. aeruginosa* by the time they reach adulthood. *P. aeruginosa* infections are associated with a rapid decline in pulmonary lung function and increased risk of morbidity and mortality. *P. aeruginosa* forms biofilms which limits efficacy of antibiotic treatment, further complicating the treatment regime. In this study, we seek to explore alternative treatments targeting *P. aeruginosa* infection in CF lungs, specifically using bacteriophages, or "phages" as a novel antimicrobial therapy. Over 75 environmental water samples were collected from freshwater ponds located around the Perth metropolitan area and assessed for presence of *P. aeruginosa* specific phage using strain PA01 (ATCC 15692). Phage isolates exhibiting anti-*P. aeruginosa* activity were propagated from 4 water samples collected. These isolates were further characterized for their stability and lytic capabilities as well as their ability to clear clinical isolates of *P. aeruginosa*. The effect of phage treatment will then be assessed by measuring bacterial load and epithelial inflammatory cytokine production following a *P. aeruginosa* infection. Generated results will give insights into the efficacy of phage therapy in CF with the potential to develop a novel therapeutic pipeline to help treat CF bacterial lung infections.

Solute Accumulation in Response to Osmotic Stress in *Acidihalobacter aeolianus*

April Gifford[1], Melissa Corbett[1], Elizabeth Watkin[1]

[1]School of Pharmacy and Biomedical Sciences, CHIRI Biosciences, Curtin University, Australia

Acidihalobacter are a unique genus of iron/sulphur oxidising bacteria capable of living in acidic/high chloride environments. The most commonly studied iron/sulphur oxidising acidophiles are used in biomining, and are notoriously intolerant of chloride, creating issues within the industry due to the large amounts of fresh water required for the metal extraction process. The purpose of our research is to investigate the mechanisms by which *Acidihalobacter aeolianus* (DSM 14174) tolerates high levels of chloride, unlike other known acidophiles. Previous genomic and protein studies have led to the hypothesis that *A. aeolianus* is utilising the accumulation of compatible solutes such as ectoine, glycine betaine and proline as osmoprotectants. However, the quantification of compatible solutes has not been studied in this species. *A. aeolianus* was grown in increasing molar concentrations of NaCl or MgSO4 then the compatible solutes were detected and quantified by mass spectrometry. Preliminary data has shown the presence of hydroxyectoine in all cultures, with betaine and ectoine also detected in significant amounts while proline was not detected in any conditions. The novelty of *A. aeolianus* creates potential for discovering unique mechanisms of stress tolerance in acidophiles, the conclusion of this research will help define potential pathways of interest for future study.

Development of a Model for Bioengineering of *Streptococcus agalactiae* Temperate Phages

Princy Shoby[1,2], Shakeel Mowlaboccus[2], Matthew Payne[1] and Lucy Furfaro[1]

[1]Division of Obstetrics and Gynaecology, The University of Western Australia

[2]Marshall Centre for Infectious Diseases Research and Training, The University of Western Australia

Streptococcus agalactiae or Group B Streptococcus (GBS) accounts for approximately 50% of significant bacterial neonatal infections. Intrapartum antibiotic prophylaxis is currently the best option against vertical transmission of GBS, however, it could negatively affect infant microbiota and could lead to metabolic disorders. Owing to their specificity, bacteriophages have been considered as an alternative treatment regimen. To date, only temperate phages have been isolated in GBS, which are not suitable for therapy. The aim of this study was to characterise a novel GBS prophage (90VΦ) and develop a model system for assessing phage-bacteria interactions. This involved curing the bacteria of 90VΦ, to obtain a compatible viable bacterial host and phage pair, as standard phage induction usually destroys the bacterial host. The host range of 90VΦ was tested against 150 perinatal GBS isolates by spot tests and whole plate assays. Phage activity was also tested against 15 different closely-related bacterial species. Virion stability was determined at different temperatures and pH conditions. Phage activity was observed against 29 (19.3%) isolates, including 50 rectal (28%), 90 vaginal (15.6%) and 10 neonatal invasive disease isolates (10%). 90VΦ was not active against species closely-related to GBS. Virions were stable at pH range 3-9 and at temperatures of 25°C, 37°C and 42°C, with no significant decline in titer. To cure the host from 90VΦ, a suicide vector was successfully constructed which would allow replacement of 90VΦ with a kanamycin-resistant marker. The characterisation of 90VΦ highlights the therapeutic potential of phages to specifically target pathogenic bacteria. The development of this model would help us to modify genes in the prophage and assess their impact on the compatible cured bacterial host

Improving the Therapeutic Value of Antimicrobial Bandages for Burn Wound Patients

Joshua P. Barrett[1,2], Joshua P. Ramsay[1,2], Ranil Coorey[1,3], Gary A. Dykes[1,4], Joshua T. Ravensdale[1,4]

[1]Curtin Health Innovation Research Institute, Curtin University, [2]School of Pharmacy and Biomedical sciences, Curtin University, [3]School of Molecular and Life sciences, Curtin University, [4]School of Public Health, Curtin University

Burn wounds are vulnerable to bacterial colonisation and if bacteria invade the circulatory system a life-threatening systemic infection may occur. Antimicrobial bandages provide a barrier over the wound, which protects against bacterial invasion. Most antimicrobial antimicrobial bandages are impregnated with inert, broad-spectrum antimicrobial compounds such as silver. However, patients suffering severe burns can develop life-threatening reoccurring infections even when wounds are bandaged. The burn wounds department at a Perth hospital have estimated that 20% of patients return with life-threatening reoccurring infections even with proper application of antimicrobial bandages. This project aims to assess the antimicrobial activity and cytotoxicity of silver nanoparticle loaded bandages (Acticoat™) combined with different formulations of wetting agents and antimicrobial compounds, such as antimicrobial peptides. The antimicrobial activity of the bandages against multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was tested on pig skin models. Skin in solidified agar that had been seeded with a bacterial concentration of 10^4cfu/cm^2 was over-laid with Acticoat bandages treated with different test formulations. Bacteria from the pig skin and over-laid Acticoat were counted following at 24h, 48h and 72h incubation at 37ºC. A human skin model will be grown using keratinocytes in Nunc cell culture insert carrier plates to test the potential cytotoxicity of the bandages. The most effective test formulations using MTT assays, scanning electron microscopy and hematoxylin and eosin staining. The results of this study could potentially help in the development of new bandages that are able to control multidrug resistant strains of bacteria on vulnerable topical wounds.

The Virulence and Pathogenicity of Lineage 4 of Dengue Virus Serotype 2, Cosmopolitan Genotype in Bali, Indonesia.

Mim Hedges[1], Allison Imrie[2], David W. Smith [3] Kritu Panta[4], Timo Ernst[4]

[1]School of Biomedical Science, University of Western Australia, [2]The University of Sydney, Sydney, New South Wales. [3]Laboratory Medicine, [4]The University of Western Australia, School of Pathology and Laboratory Medicine

Introduction -The epidemic potential of dengue virus (DENV) serotypes (1-4) remains poorly defined. The superior epidemiological fitness of specific lineages has been attributed to a spectrum of host and viral factors. The evolution of DENV 2 in South East Asia was shaped by selection pressures that conferred enhanced virulence governing genotype replacement events. High viremia is correlated with greater transmission potential. Levels of viremia are subject to phenotypic properties of the infecting virus as well as the efficiency of the host immune response. These factors evolve simultaneously making it difficult to determine whether clade replacement events are attributed to host or viral factors. To successfully control outbreaks it is necessary to understand the mechanisms that govern the epidemiology of DENV existing in any ecological niche. **Problem statement** -In Bali, Indonesia during 2010 – 2013 a new Lineage (L4) of the cosmopolitan genotype of DENV 2 emerged and remained dominant. It is unclear why this lineage persisted while others dropped out. **Methods** - Seven lineages of DENV 2 that represent the spectrum of circulation dynamics in Bali between the years 2010 and 2013 were grown in a Vero cell line. Supernatants were harvested at sequential time points and an enzyme linked focus forming assay was performed to characterise the replication kinetics of each lineage. **Results** L4 produces early CPE and replicates faster than the other 6 prototype viruses. Insight into the phenotypic properties of L4 may contribute an understanding for the mechanisms underlying its persistence. To determine whether L4 induces a comparable cytokine response to the other prototype viruses a multiplex cytokine assay will be employed to characterise the cytokine profiles of virally infected PMBC's. By comparing both replication kinetics and cytokine profiles of each lineage, I will provide further evidence to indicate whether the evolutionary advantage of L4 can be attributed to its unique phenotypic properties.

Investigating the Role of Peptidoglycan Recycling in the β-lactam Resistance of *Burkholderia cepacia* Complex Species

Rebecca Kaard[1], Keith Stubbs[2], Aleksandra Debowski[1], Mitali Sarkar-Tyson[1]

[1]Marshall Centre for Infectious Disease Research and Training, School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia. [2]School of Molecular Sciences, University of Western Australia, Perth, WA, Australia.

Several bacterial species within the *Burkholderia cepacia* complex (BCC), most notably *Burkholderia cenocepacia*, are important opportunistic pathogens. These species are of significance to individuals suffering from Cystic Fibrosis, where infection can lead to the development of 'cepacia syndrome', a sudden, uncontrollable clinical decline, resulting in death. It's becoming increasingly difficult to treat BCC infections on account of their extensive antibiotic resistance. One of the main mechanisms of resistance is the production of β-lactamases, encoded by genes such as *ampC*, which can inactivate cephalosporins, one of the few antibiotics still effective at treating BCC infections. NagZ, an enzyme in the peptidoglycan recycling pathway, is responsible for the liberation of the metabolite that induces *ampC* transcription. Studies using *Pseudomonas aeruginosa* have shown that blocking the activity of NagZ prevents the induction of AmpC β-lactamases and can make normally resistant *P.aeruginosa* strains more susceptible to β -lactam antibiotics. One study has shown that a similar regulatory mechanism may also control the expression of two β-lactamase genes in *B. cenocepacia*. The aim of this work is to investigate if NagZ is involved in the regulation of β-lactamases in *B. cenocepacia* and validate it as a target for therapeutic intervention. This involves generating and characterizing a *B. cenocepacia nagZ* mutant and its respective complement, evaluating the level of β-lactam resistance in BCC clinical isolates, and test if inhibition of NagZ can block the induction of AmpC-β-lactamases, thereby making *B. cenocepacia* more susceptible to β-lactam antibiotic therapy. Minimum Inhibitory Concentration (MIC) assays performed on the *nagZ* deletion mutant show increased levels of β-lactam susceptibility in comparison to wild type. A *nagZ* complementation construct has been created and is currently being characterized. Validating NagZ as an effective target for blocking β-lactamase production will thereby assist in the treatment of BCC infections using β-lactam antibiotics.

Developing a Frontline Treatment for Neonatal Sepsis

Breanna Knight[1], Tobias Strunk[2,3], Sam Abraham[1], Rebecca Abraham[1] and Andrew Currie[1,3]

[1]Medical, Molecular & Forensic Sciences, Murdoch University, Perth, WA, [2]Neonatal Directorate, King Edward Memorial Hospital for Women, Perth, WA, [3]Neonatal Health and Development, Telethon Kids Institute, Perth,WA

Preterm infants are highly susceptible to neonatal sepsis with substantial morbidity and mortality. Early empiric treatment is mandatory as blood cultures results take up to 48 hours. Empirical treatment is ineffective if the wrong pathogen is targeted. Antimicrobial peptide IDR1018 is a novel adjuvant antimicrobial with broad activity. Importantly, IDR1018 may synergise with common neonatal antibiotics. Antibacterial checkerboard assays were performed of IDR1018 and the neonatal antibiotics vancomycin or gentamicin against prototypical neonatal pathogens. Standard testing conditions used RPMI1640 + 5% Luria Bertani broth. Further, time-to-kill assays were performed with samples taken at 0, 2, 4 and 24 hours to determine the effectiveness and kinetic of the drug combination in adult human serum, and compared against individual antibiotics or IDR1018 only and the empiric combination of gentamicin and vancomycin. Synergy was observed between vancomycin and IDR1018 against 5 clinical isolates of *E. coli* and 1 ATCC strain using the Fractional Inhibitory Concentration Index. No synergistic activity was observed in human serum IDR1018 and vancomycin against *E. coli* and *S. aureus*. IDR1018 had no activity against the bacteria independently. IDR1018 can synergise with the commonly used antibiotic vancomycin, against both Gram negative and Gram positive neonatal pathogens under standard testing conditions. IDR1018 was inactive against *E. coli* and *S. aureus* in human serum at the synergistic dose, suggesting that at low levels of the antimicrobial peptide there is a component of the serum inhibiting its activity. Further tests are required to find the component of the serum that is inhibiting activity, such as denaturing the proteins in the serum before repeating the time to kill assay, or attempting to increase the level of free IDR1018 in the serum by raising the IDR1018 dose.

I11

Characterisation of virulence determinants of hyper-virulent *Neisseria meningitidis* serogroup W clonal complex 11 isolates (MenW:cc11)

Nicolie R. McCluskey[1], Edward C. Mikucki[1], Shakeel Mowlaboccus[1], Charlene M. Kahler[1]

[1]Marshall Centre for Infectious Disease Research and Training, School of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Western Australia

Neisseria meningitidis clonal complex 11 (cc11) is a threat to public health on a global scale given its hyperinvasive nature and pathogenic potential. The cc11 lineage is associated with multiple capsule serogroups including: B, C, and W (MenW), and has been the cause of numerous invasive meningococcal disease (IMD) outbreaks globally. In 2016, MenW:cc11 became the predominant cause of IMD in Western Australia, representing 67% of all IMD cases reported that year. Previous studies from our laboratory have shown that the invasive potential amongst the hyperinvasive meningococcal genetic lineages is widely variable even when all strains tested have been selected for the expression of the same major virulence determinants. In 2013, it was shown in attachment and invasion assays into Detroit 562 epithelial cells that a MenB:cc8 isolate was 20-fold more invasive than a MenB:cc32 isolate. A subsequent study in 2018, using the same infection assays, showed that MenW:cc11 isolates circulating in WA were 10- to 20-fold more invasive than the MenB:cc8 strain. However, the factors contributing to variation in invasive potential remain unknown. This study aims to: (1) characterise and assess the roles of known meningococcal virulence factors associated with the outer membrane and polysaccharide capsule on the invasive potential of three MenW:cc11 isolates from WA; and (2) assess their contribution to attachment and invasion into Detroit 562 epithelial cells. The major virulence factors under assessment include the expression of the polysaccharide capsule, type IV pilus, pilin glycosylation, LOS sialyation, and the MisR/S two component regulatory system. Virulence gene knockout mutants have been constructed using either natural or chemical transformation and will be assessed using the Detroit 562 invasion model. We hypothesise that the major virulence factors will play a role in MenW:cc11 isolates invasion into Detroit 562 epithelial cells.

I12

Re-Evaluating the Role of Restriction Modification Systems as a Barrier to Horizontal Antimicrobial-Resistance Gene Transfer in *Staphylococcus aureus*

Amy Davis[1], Karina Yui Eto[1], Geoffrey Coombs[2], Neville Firth[3] and Joshua Ramsay[1]

[1]Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University. [2]School of Veterinary and Life Sciences, Murdoch University. [3]School of Life and Environmental Sciences, University of Sydney

Staphylococcus aureus is a highly clonal species and can be divided into lineages – or clonal complexes – based on point mutations in the core genome. Most lineages carry one or more distinct variant of a Type I restriction-modification (RM) system (HsdR) and these are generally viewed as a major barrier to horizontal gene transfer (HGT) between lineages. Indeed, deletion of *hsdR* genes greatly increases the efficiency of DNA electroporation in laboratory experiments. To evaluate the extent that these RM systems disrupt natural HGT mechanisms, conjugation experiments and bacteriophage plaque assays were carried out using RM proficient and deficient strains. Representatives of three distinct conjugative plasmid families were transferred to wildtype and RM-deficient mutants of the three community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains MW2, MRSA252 and JKD6159. pSK41 and pWBG749-family conjugative plasmids exhibited high rates of conjugative transfer (10^{-2}-10^{-3}) to all three CA-MRSA strain. Less than a 10-fold increase was observed for conjugation to the RM-deficient strains, which in most cases was not statistically significant. Only the pWBG4-family conjugative plasmid exhibited a 100-fold increase in transfer to the RM-deficient derivative of JKD6159, which carries deletions in two RM systems. Bacteriophage plaque assays with Phage K revealed the RM systems inhibited plaque formation around 100-1000-fold, but did not abolish phage infection. These observations conflict with the generally accepted role of RM systems as a major barrier to gene transfer in *S. aureus*. We propose that RM systems do not present a complete barrier to gene transfer in *S. aureus* to and that they are particularly ineffective in blocking the most common conjugative plasmids responsible for dissemination of antimicrobial resistance.

The Nuclear Receptors at the Interface of the Gut Microbiota-Host Communication

Patrice Maher[1,2], Jose A. Caparros-Martin[1,2], Fergal O'Gara[1,2,3]

[1]Human Microbiome Programme, School of Pharmacy and Biomedical Sciences, Curtin University, Australia, [2]Curtin Health Innovation Research Institute (CHIRI), Curtin University, Australia, [3]BIOMERIT Research Centre, School of Microbiology, University College Cork Ireland.

Communication is essential in living organisms. This fact is critical in the case of ecological communities, in which communication allows adaptation in response to environmental changes. The human body is home of complex communities of microorganisms, which are referred as to the human microbiota. In the recent years, evidence has continued to emerge on the role of these communities in the maintenance of human health and the development of metabolic disorders such as diabetes and cardiovascular disease. A necessity has emerged in favour of identifying the molecular pathways and mechanisms regulating the crosstalk host-microbiota in homeostatic conditions, in order to develop targeted interventions to reverse the microbiota-mediated disease-related phenotypes. Our recent research has highlighted the importance of a group of proteins called nuclear receptors, in regulating both human and microbiota metabolism. In this project we have gained insight into how this regulation occurs. Using mice models (wild type and double knock-out in each treatment group), we have investigated how activation of this group of proteins in the liver affects host-microbiota homeostasis. Processing the genetic material from our models through gut microbiota profiling and analysis as well as conducting a functionality profile enabled a broader understanding of the possible effects of activation of these proteins. Our preliminary results indicate that there is a correlation between activation of nuclear receptors and physiological changes in the host. This systemic response also can be associated with both changes in the functionality of the gut microbiota and alterations of the metabolism in the host. Our data suggests that modulation of the activity of these nuclear receptors in the liver could promote specific gut microbiota functional profiles to reinforce their effect on host metabolism.

Comparative Genomics of Cholesterol Metabolism Genes in *Vicugna pacos*

Jamie-Lee Pitcher[1], Mark Agostino[1], Cyril Mamotte[1], Kylie Munyard[1]

[1]Curtin Health and Innovative Research Institute, School of Biomedical Sciences, Faculty of Health, Curtin University, Perth, Western Australia

Lipid metabolism is a complex and intricate process that is not yet fully understood in humans. With lipid metabolism-associated conditions, such as type 2 diabetes, rising in prevalence every year, the need to find treatments and ameliorating agents is crucial. While useful, current animal models are limited in their translative capacity. New models in large mammals, such as *Vicugna pacos* (alpacas), have great potential to bridge this gap due to their unique evolutionary relationship to humans. Alpacas are used globally for agriculture, making them accessible, and research in this species may benefit both human lipid metabolism research and the alpaca industry. The lipid metabolism of alpacas has not yet been explored; however, they have a unique glucose metabolism that makes them constantly hyperglycaemic without clinical signs of diabetes. Given the association between glucose and lipid metabolism it is plausible that alpacas have an abnormal lipid metabolism, making them potentially suitable as a model for lipid and glucose related conditions. Cholesterol is an essential lipid with diverse functions associated with many lipid metabolism-related conditions, and so genes linked to cholesterol metabolism were targeted in this study. Functional domain, phylogenetic, selection signature and signal peptide analyses were conducted on cholesterol metabolism protein sequences from alpacas, humans and three current lipid metabolism models; mouse, pigs and dogs. The functional apolipoprotein domain for apolipoprotein E that is present in humans was absent in the alpaca protein and significant phylogeny was observed between humans and alpacas for CCHC-type zinc finger nucleic acid binding protein and NAD(P) dependent steroid dehydrogenase-like. These results indicate that alpacas may be a viable animal model for cholesterol metabolism, however, further research both *in silico* and *in vitro* is required to confirm these findings.

Innate Immune Responses of Cystic Fibrosis Airway Cells to Rhinovirus Infection

Ling kak-Ming[1,2], Garratt Luke W[2], Agudelo-Romero Patricia[2], Sutanto Erika N[2], Iosifidis Thomas[2], Gill Erin E[3], Lee Amy[3], Turvey Stuart E[4]. Hancock Robert E[3], Lassmann Timo[2], Kicic Anthony[1,2,5,6], and Stick Stephen M[1,2,6], on behalf of WAERP[1,2,6,7] AREST CF[2,6,8,9]

[1]UWA [2]Telethon Kids Institute [3]Microbial Diseases Research & [4]Pediatrics, University of British Columbia [5]Public Health, Curtin University [6]Resp and Sleep Med, PCH [7]St. John of God Hospital [8]Murdoch Children's Research Institute [9]Paediatrics, University of Melbourne

Viruses are the predominant respiratory infection in early life and can cause exacerbations in CF. Prior studies of rhinovirus (RV) infection in primary CF airway epithelial cells (pAEC) have largely explored inflammatory and antiviral pathways. Whether virus infection contributes to other airway pathologies in CF such as abnormal mucus accumulation is unknown. We hypothesised that the antiviral response of pAEC from children with CF are dysregulated following RV infection. To address this question, we compared transcriptional responses to RV infection between non-CF and CF pAEC.pAEC cultures from children with CF (n=7) and non-CF controls (n=5) (age; all <5 years) were infected with RV, RNA extracted, and RNA-sequencing performed (Illumina Hi-Seq2500). Sequence reads were mapped to the human reference genome using HISAT2 and gene counts were performed using SummarizeOverlaps. Differential gene expression (DEG) analysis was performed using DESEq2 to identify the transcriptomic responses of non-CF and CF pAECs to rhinovirus infection. We identified 877 DEGs in non-CF (613 upregulated and 238 downregulated) and 1379 DEGs in CF (828 upregulated; 525 downregulated) pAECs in response to RV infection, predominantly, relating to interferon and inflammation pathways. A total of 21 unique biological pathways were identified in CF. One non-canonical enriched pathway of interest was O-linked glycosylation of mucin. Specifically, genes encoding n-acetylgalactosaminyltransferases and sialyltransferases (*GALNT10, GALNT11, ST6GALNAC1, ST6GALNAC2, ST6GALNAC5*) were downregulated (1.6-2 fold) while n-acetlyglucosaminyltransferase and galactosyltransferases (*B3GNT8, B4GALT1, B4GALT5)* were upregulated (1.8-1.9 fold). This was accompanied by increased expression of membrane-tethered mucin genes (*MUC1, MUC15*; 3.2-4.4-fold). We are currently assessing the impact of RV on secretory mucin expression (MUC5AC and MUC5B) in differentiated pAEC cultures. CF pAEC elicit more complex responses upon virus insult than previously thought. Changes in glycosyltransferases gene expression could affect mucin production and secretion, suggesting a mechanism by which viral infection can affect the airway microenvironment early in life

J3

Circulating Exosomal miRNA for Predicting Pregnancy Outcome in High Risk Women: An Optimisation Study

Julian Y Hou[1], Blagica Penova-Veselinovic[1], Carlos Salomon[2], Jeffrey A Keelan[1]

[1]Faculty of Health and Medical Sciences, The University of Western Australia, [2]Exosome Biology Laboratory, Centre for Clinical Diagnostics, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, The University of Queensland

Introduction/Problem. Preterm birth (PTB) is defined as parturition before 37 weeks' gestation. It affects 12% of pregnancies globally (8.6% of Australian births) and is associated with high risk of perinatal mortality and morbidity. Current clinical assessments/screening tests for PTB (e.g. cervical length, fetal fibronectin) either have low sensitivity/specificity or are unsuitable for use in early pregnancy. Several studies have shown an association between altered expression of miRNA within small extracellular vesicles called exosomes in maternal circulation and PTB risk; however, scalable methods are required to establish the clinical predictive utility of exosomal miRNA measurements in pregnancy. **Objectives.** The overall aim of this project is to explore diagnostic potential of circulating exosomal miRNAs in maternal serum for predicting preterm birth risk and response to treatment in high-risk women. Here we have evaluated and optimised methods for exosome isolation and miRNA extraction from maternal serum for use in future miRNA profiling studies. **Methods.** Exosomes were purified from maternal sera (n=3) (WA Pregnancy Biobank) at three gestational ages (~12, 18 and 28 weeks) using a polymer-based precipitation method (miRCURY), comparing a 1-hour *vs*. overnight protocol. Nanoparticle tracking analysis (NTA) was performed to measure exosome concentration and size distribution. RNA was extracted using the miRNeasy kits (Qiagen), optimised for either total RNA (>200 nt) or enriched miRNA extraction; miRNA enrichment was assessed using capillary electrophoresis (2100 Bioanalyzer). **Results**. NTA results showed that the proportion of exosomes (~35-125 nm) in total microvesicle fraction increased as pregnancy progressed, consistent with previous publications. Increased precipitation time did not improve the exosome yield or quality. Bioanalyzer results showed no significant improvement in miRNA enrichment with different extraction protocols. **Conclusions**. One-hour exosome precipitation is equally as effective as overnight precipitation. Purifying total RNA in opposed to enriching the miRNA fraction allows for faster extraction with similar miRNA yields.

J4

Platelet Activation Pathways are Disrupted During Acute Anaphylaxis in Humans

Francesca McGrath[1,2], Andrew Woo[3], Daniel Fatovich[1,2,4], Glenn Arendts[1,2,5], Erika Bosio[1,2]

[1]Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research, [2]Division of Emergency Medicine, UWA, [3]Laboratory for Cancer Medicine, Harry Perkins Institute of Medical Research, [4]Emergency Department, RPH, [5]Emergency Department, FSH

Introduction. Platelets characteristically mediate hemostasis and thrombosis, however, they may also play a role in anaphylaxis as demonstrated in mouse models, and most recently in humans. **Problem Statement**. Despite this recent implication, little is known about the activities of platelets in human anaphylaxis. **Procedures**. Microarray analysis was performed on whole blood samples (Paxgene) collected from 10 healthy controls and 18 patients during acute anaphylaxis. Differentially expressed genes (DEGs) were grouped at different time points (ED presentation and 1 hour post-enrolment), severities (moderate and severe), and triggers (food and drug) and the enriched pathways were analysed. Genes involved in the Reactome Pathway for platelet activation, signaling and aggregation were downloaded from the Comparative Taxicogenomics Database (CTD) and compared to DEGs from the microarray analysis. Protein-protein interactions (PPIs) of DEGs involved were constructed using STRING and Cytoscape. **Results**. While genes involved in platelet activation, signaling and aggregation were dysregulated across all groups, more genes involved in platelet pathways were dysregulated in severe (24 genes) and drug-triggered reactions (30 genes) compared to moderate (5 genes) and food-triggered reactions (7 genes). Little difference in these platelet pathways was seen between the two timepoints (30 genes time 0, 32 genes time 1). A core set of 4 genes; SELP (adjp<0.04), GP1Ba (adjp<0.04), F13A1 (adjp<0.04), and SPARC (adjp<0.04), were downregulated in all anaphylaxis groups compared to healthy controls. **Conclusions**. These results suggest platelets are involved in multiple types of anaphylactic reactions, and that dysregulation of 4 key genes may drive their involvement. Additionally, increased platelet disruption appears associated with severe reactions. These newly identified key genes may serve as an important marker of anaphylactic reactions and give insight into how platelets influence reaction progression.

J5

Genome-scale Phylogeny and Evolutionary Analysis of Ross River virus Reveals Periodic Sweeps of Lineage Dominance in WA, 1977 – 2014

Alice Michie[1], Vijaykrishna Dhanasekaran[2], John S. Mackenzie[3], David W. Smith[3], Allison Imrie[1,3]

[1]School of Biomedical Sciences, University of Western Australia, Western Australia, [2]Biomedicine Discovery Institute, Monash University, Victoria, [3]PathWest Laboratory Medicine Western Australia

Ross River virus (RRV) is the most medically significant mosquito-borne virus of Australia, with an average 5000 clinical cases reported per annum. In Western Australia (WA), surveillance of RRV is based on the isolation and identification of viruses from the homogenate of pooled and trapped mosquitoes. Routine and opportunistic mosquito trapping for surveillance purposes has been conducted throughout WA since the mid-1980s. Isolates have been classically identified using monoclonal antibodies, and more recently, RT-PCR. Past RRV phylogenetic and evolutionary analyses have been based on a small collection of partial genomes only. Three geographically distinct RRV lineages: the Eastern, the Western and the supposedly extinct North-Eastern lineage have been classified. We sought to expand on past phylogenies through robust genome-scale analysis to better understand RRV genetic diversity and evolutionary dynamics. We analysed 106 RRV whole genome sequences which included 12 publicly available sequences and 94 novel sequences derived for this study, sampled throughout WA (1977 – 2014) and during the substantial Pacific Islands RRV epidemic (1979 – 1980). Our final dataset comprised isolates sampled over a 59 year period (1959 – 2018), from a range of locations. Four distinct genotypes (G1-4) were defined, with the newly described G4 found to be the contemporary lineage in circulation. The prior geographical classification of RRV lineages was not supported by our findings, with evidence of geographical and temporal co-circulation. Bayesian Markov Chain Monte Carlo (MCMC) analysis revealed that RRV lineages diverged from a common ancestor approximately 94 years ago, with distinct lineages emerging roughly every 10 years over the past 50 years in periodic bursts of genetic diversity. Our study has enabled a more robust analysis of RRV evolutionary history, and resolved greater genetic diversity than previously defined by partial genome analysis.

J6

Secretome of Human Mesenchymal Stem Cells for Wound Healing

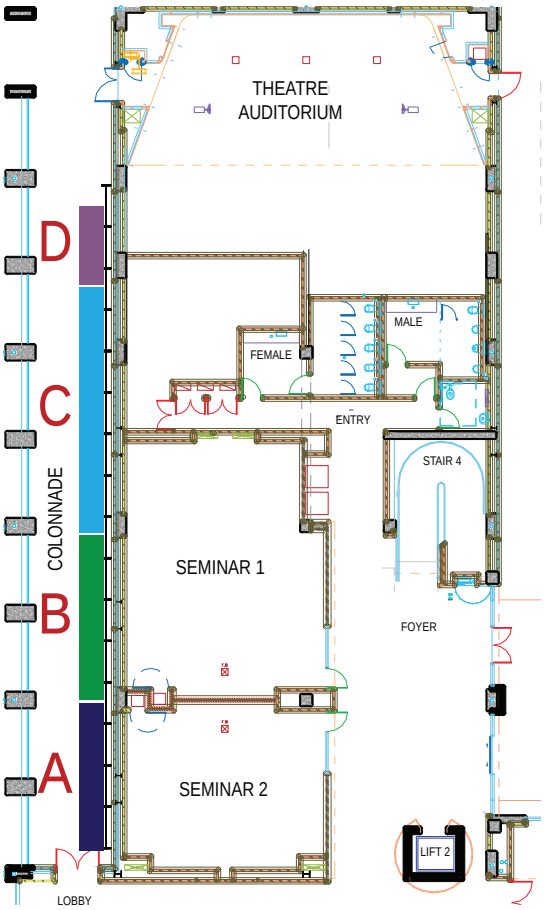
H.T. Ong[1,2], S.L. Redmond[1,2], D.B. Vargas-Landin[3], R. Roy[4], A. Forrest[4], R.J. Dilley[1,2,5]

[1] Ear Science Institute Australia, [2] Ear Sciences Centre, University of Western Australia (UWA), [3] School of Molecular Sciences, UWA, [4] Harry Perkins Institute of Medical Research, [5] Centre for Cell Therapy and Regenerative Medicine, UWA

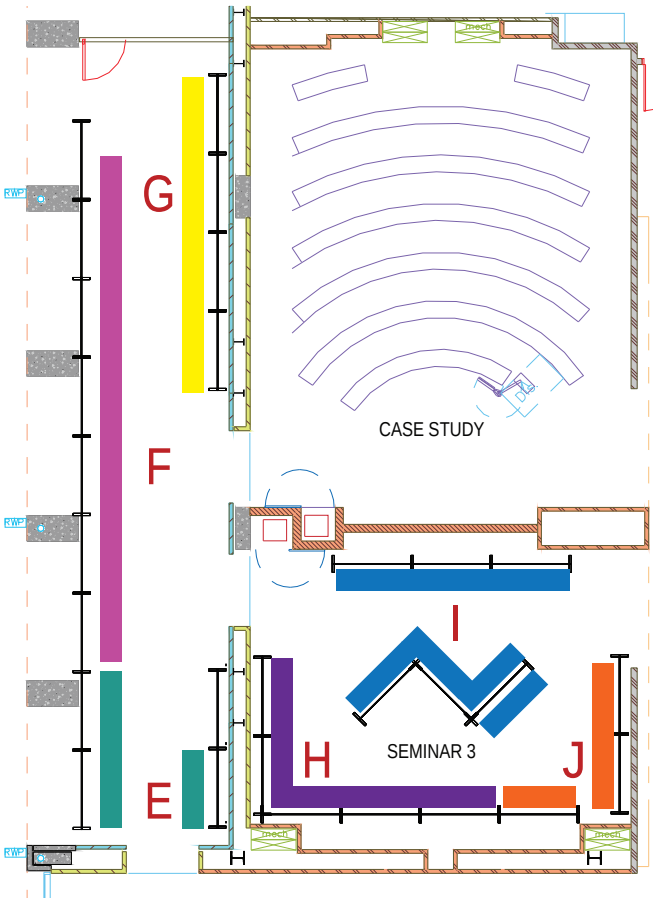
Introduction. Our studies have previously shown that human adipose-derived mesenchymal stem cells (ADSC) produce an activity that stimulates wound healing in eardrum keratinocytes (hTMk). **Problem Statement.** The molecular mechanism behind this paracrine activity of ADSCs on wound healing is not known so we have used RNAseq with a bioinformatics approach to determine the transcriptome profile of ADSC and hTMk for secreted ligands and receptors. **Procedures**. ADSC were cultured in either ambient oxygen conditions (21%) or in a hypoxia chamber (<0.1% O_2) for 48 hours without serum. hTMk cultures were established under normoxic conditions. Conditioned media (CM) were collected from ADSC to assess paracrine activity on hTMk proliferation and migration, and to quantify specific protein secretion using ELISA. Transcriptomic analysis of ADSC and hTMk were assessed using RNAseq and bioinformatics analysis. Transcripts differentially expressed (adj-P<0.05) between hypoxic and normoxic ADSC were filtered through databases to identify secreted ligands, . and similarly for hTMk transcriptome to identify receptors. Both subsets were then matched to a ligand-receptor pair database curated with the FANTOM5 consortium for a final set of ligand-receptor repertoires between ADSC and hTMk. A final target set was then put through Gene Ontology analysis to identify crucial pathways involved. **Results.** A total of 492 ligand-receptor pairs with 166 ADSC ligands and 166 hTMk receptors were identified. Of the 492 pairs, 90 were ligands upregulated >2-fold by hypoxia, 219 down-regulated, 42 turned on, 45 turned off, and 96 had less than 2-fold change in hypoxia. Paracrine activity produced by ADSC under hypoxic conditions showed enhanced wound healing for human eardrum keratinocytes *in vitro*. **Conclusions**. Bioinformatics analysis was able to identify and predict the potential paracrine effectors for this wound healing activity. Further experiments are necessary to test and verify potential molecular mechanisms behind wound healing effects of ADSC on eardrum keratinocytes.

Poster Locations

POSTERS: GROUND FLOOR
LOWER COLONNADES



POSTERS: FIRST FLOOR
UPPER COLONNADES & SEMINAR ROOM 3



SECTION	TOPIC	POSTER AWARD SPONSOR
A	Biochemistry & Molecular Biology	ASBMB
B	Health Sciences	CBSM
C	Senior Research Presentations	John Morris Scientific
D	Plant Science	CBSM
E	Cell & Developmental Biology	ANZSCDB
F	Immunology	ASI
G	Neuromuscular & Neuroscience	Perron Institute
H	Genetics	HGSA
I	Microbiology	ASM
J	~Omics	Murdoch University

Poster Presentation Index

AUTHOR	POSTER	
Alves	E	H8
Andradas Arias	C	C9
Audsley	K	F8
Aung	S	F7
Aung-Htut	M	C6
Baklous	L	H10
Bamb	K	I2
Barrett	J	I7
Bennett	S	B4
Bieniawski	M	A1
Black	A	H7
Boulter	J	E4
Brace	A	D4
Brown	R	E1
Buck	J	C2
Byrne	J	F1
Caparros-Martin	JA	C7
Castro Suarez	J	F12
Chakraborty	A	D3
Chen	S	A7
Chen	K	B3
Cilemanoff	S	A5
Dart	S	F9
Davis	Al	E6
Davis	Am	I12
Debler	J	C8
Gearon	F	B5
Gifford	A	I5
Gorecki	A	G3

AUTHOR	POSTER	
Greig	H	F5
Ha	P	G6
Haese	E	I1
Harfield	J	E3
Hedges	M	I8
Hii	H	C1
Hou	L	D2
Hou	J	J3
Huang	D	H3
Huynh	T	C10
Kaard	R	I9
Khandelwal	A	I3
Knight	B	I10
Lareu	R	C12
Laucirica	D	F6
Li	D	G1
Li	Y	H9
Ling	K	J2
MacDougall	G	A6
Maher	P	I13
Martinovich	K	A4
McCluskey	N	I11
McGrath	F	J4
McIntosh	C	G7
Mejzini	R	G2
Michie	A	J5
Motteram	L	B1
Ng	R	I4
Ong	HT	J6

AUTHOR	POSTER	
Orozco	L	B6
Ouliel	E	G4
Oyemade	M	F4
Parameswari Thanigaiarasu	L	G5
Paxman	A	E2
Pitcher	J	J1
Pitout	I	C11
Poudel	BH	G8
Pytte	J	H4
Raymundo	M	B8
Rekima	A	C3
Ribet	A	E5
Rwandamuriye	FX	F10
Safaeefar	S	F11
Shoby	P	I6
Slater	N	F13
Strowger	B	C5
Tahghighi	H	D1
Tan	YH	B7
Thomas	E	F2
Tongpob	Y	B2
Utama	S	H2
van den Elsen	L	C4
Waters	J	H1
Weston	B	F3
Witham	C	A2
Witham	C	A3
Zaw	K	H5
Zimmerle	S	H6

Combined Biological Sciences Meeting 2019

Time	Session	Speaker	Title	Chair
8-8:40	Early Career Researcher Development Session (Case Study Room)	Dr Joshua Lewis	Writing a successful fellowship application, lessons learned the hard way!	Dr Raelene Endersby Ms Hannah Greig
8-8:30	**Registration and Breakfast**			
8:45	Welcome	Dr Clayton Fragall	Opening address	
9:00	Plenary (Theatre Auditorium)	Professor Sarah Bekessy	The critical role of 'everyday nature' for the future of cities	Dr Charlotte Oskam
9:40	Dept of Heath WA New Investigator Session (Theatre Auditorium)	Ms Shelley Waters Ms Jennifer Currenti Dr Lucy Furfaro Dr Rachael Zemek	Digging deep to reveal novel CMV variants and associations with patient outcome Deep sequence analysis of HIV adaptation following vertical transmission reveals the impact of immune pressure on the evolution of HIV Group B Streptococcus colonisation of Western Australian pregnant women Sensitisation to immune checkpoint blockade through activation of a STAT1/NK axis in the tumour microenvironment	Dr May Aung-Htut Dr Jessica Buck
	ECU New Investigator Session (Case Study Room)	Mrs Julie Sartori Dr Kritu Panta Dr Kai Chen Dr Pauline Zaenker	The Placenta Project: Characteristics in Assisted & Non-Assisted Pregnancy for a Western Australian Cohort Dengue antibody and Zika Pseurotin A Prevents Estrogen Deficiency-induced Bone Loss by Inhibiting Osteoclastogenesis Identification and Validation of a Diagnostic Autoantibody Signature for Primary Cutaneous Melanoma	Dr Megan Lloyd
10:40	**Morning Tea (sponsored by Murdoch University)**			
11:00	Upper and Lower Colonnade	**Poster Session**		
11:30	Plenary (Theatre Auditorium)	Professor Mark Nicol	Early life microbial exposures and health outcomes in young African children	Assoc Prof Charlene Kahler
12:10	Enabling Technologies Session (Theatre Auditorium)	Dr Torben Kimhofer	The Australian National Phenome Centre	Mr Lawrence Liew
12:30	**Lunch (sponsored by Telethon KIDS Institute)**			

		Australian Society for Microbiology (Theatre Auditorium)	**Cell and Developmental Biology** (Case Study Room)	**Frontiers in Genetics** (Seminar Room 1)	**Biodiversity** (Seminar Room 2)
1:30	Keynote Speakers	Professor Helen Marshall Impact of MenB vaccine on meningococcal disease and carriage of N. meningitidis in S. Aust. Adolescents	Dr Michael Lazarou Sending bad mitochondria to the garbage disposal: Insights into PINK1/Parkin mitophagy	Dr Sam Abraham Seagulls and Superbugs: Role of genomics in understanding the spread of antimicrobial resistance	Dr Nathalie Butt Tough choices: assessing threats and prioritising conservation investment
2:10	Student Speakers	Amy Davis Patrice Maher Emily Kibble Aleesha Davis	Janya Grainok Samuel Montgomery Emma Panting Heng Qiu	Jessica Cale Adriana Foster Laetitia Hughes Jessica Cheng	Alice Michie Kamil Braima Siobhon Egan Kenny Choo
3:10	**Afternoon Tea**				
3:30	Invited Speakers	Professor Jeffrey Keelan Assoc Professor Asha Bowen	Dr Sébastien Malinge Dr Yu Yu	Dr Christian Pflüger Ms Sarah Beecroft	Dr Jennifer Kelley Dr Alison Ritchie

4:30	Plenary (Theatre Auditorium)	Professor Alpha Yap	Tissue forces and epithelial homeostasis	Assoc Prof Nathan Pavlos
5:10	Scientific Awards	Dr Clayton Fragall	Scientific award ceremony and closing address	
5:30 - 7:30	**Sundowner (sponsored by WRAYS)**			

New Investigator Symposia

Specialist Symposia