# POSTER ABSTRACTS

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| A | Biochemistry & Molecular Biology | ASBMB |
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# Development of Novel Aptamers Specific to Mesothelioma Cells for Targeted Drug Delivery Applications

Milena Fratini[1], Rakesh N. Veedu [1,2]

[1]Centre for Comparative Genomics, Murdoch University, [2]Perron Institute Neurological and Translational Science

**Introduction**. Nucleic acid aptamer technology has attracted considerable attention in recent years in life sciences. Aptamer can bind to their target with very high affinity and specificity because of their ability to adopt three-dimensional structures, and this characteristic make them attractive molecules for disease specific drug delivery. Malignant pleural mesothelioma is a pulmonary cancer, caused by asbestos exposure. It is very aggressive and can lay dormant for 20-40 years, though once detected is deadly within a year. **Problem Statement**. Nowadays there are many different anticancer therapies. However, there are various limitations such as such as poor delivery efficacy and side effects from chemotherapy, which drive us to look into other alternatives approaches. Although there are antibodies-based methods currently used for targeted cancer therapy, there are some limitations associated with this approach such as the requirement of living system for production, batch-to-batch variation, high immunogenicity and toxicity and shorter shelf-life. Alternatively, development of nucleic acid aptamers specific to mesothelioma cells could be one approach to achieve tissue specific cancer drug delivery and therapy. Aptamers possess some advantages such as easy laboratory production in large scale, low cost, high stability, no immunogenicity and no or low toxicity. **Procedures.** Cell-Selex methodology was used to develop aptamers specifically to AE17 mouse mesothelioma cells. Therefore, a 81mer DNA library was incubated with AE17 cells to isolate internalised aptamer candidates with high affinity. Seven rounds were performed. Negative selection steps were also performed using other cells to remove any non-specific binders. **Results.** Flow cytometry analysis data suggested that the selected aptamers candidates after seven rounds of selection showed a slight shift compared to the library control. **Conclusions.** We have performed seven rounds of Cell-Selex procedures and the isolated DNA aptamer products are currently being sequenced to identify the individual internalised aptamers specific to AE17 cells.



# Uncovering the Mechanisms of Ciclopirox Olamine: an Anti-fungal Drug Repurposed for the Treatment of Metastatic Melanoma

Conrad Hogg[1,2], Rikki Brown[2], Andrew Woo[2], Peter Leedman[2]

[1]School of Biomedical Sciences, University of Western Australia, [2]Laboratory for Cancer Medicine, Harry Perkins Institute of Medical Research

Metastatic melanoma is an area of unmet clinical need, with less than 20% of patients surviving five years from their diagnosis. The standard of care is limited by the development of resistance to therapies, and the inability of many patients to respond to targeted or immunotherapies. Consequently, new treatments that can act alone or in combination with current therapies are urgently needed. Ciclopirox olamine (CPX) is an antifungal agent that has recently shown promising anti-cancer effects in phase I clinical trials of blood cancers, thus it can potentially be rapidly repurposed to treat metastatic melanoma. This study aims to uncover the underlying mechanisms of the action of CPX in melanoma by investigating the cytotoxicity of the drug *in vitro* and examining RNA and protein expression after treatment with the drug, or knockdown of pathways usually altered by CPX. While the mechanism of action of this drug remains unknown in melanoma, this study establishes that the action of CPX is strongly iron-dependent. It does not mediate its cytotoxic effect through reactive oxygen species or the Wnt/β-catenin pathway, which have previously been proposed as putative mechanisms in the literature. Furthermore, in a cell line with a BRAF mutation, CPX induces autophagy, which may contribute to the drug's mechanism of action. CPX also induces the expression of the immune cell attracting chemokines including CCL3, suggesting that CPX has the potential to be immunogenic. Thus, CPX shows promise as both a monotherapy, and in combination with immunotherapy. Future work should focus on effect of chelating iron from essential enzymes in the cancer cells such as eIF5A and ribonucleotide reductase, as well as the assessing synergy of the drug in combination with immunotherapy *in vivo.*

# Insights from Magnetofection Agent Development

Kerr, R.H.[1], Evans, C.W.[1], Kretzmann, J.A.[1], Clemons, T.D.[1], Iyer, K.S.[1]

[1]The University of Western Australia

Magnetofection describes transfection protocols that rely on magnetic materials associated with gene delivery agents to provide targeting and amplified action through interaction with an external magnetic field. Improvements in DNA delivery have relevance for future adjuvants in the treatment of cancer, the first world's leading cause of mortality, in gene therapy, and preparation of transformed cells in research. It was expected that a magnetofection agent consisting of superparamagnetic iron oxide nanoparticles (SPION) and an existing high-performance transfection agent could utilise an external magnetic field to achieve effective transfection under challenging conditions such as co-transfection, or short transfection timeframes. Transfection agents were electrostatically associated with SPION and used to deliver a GFP encoding 5.3kb reporter plasmid to immortalised cell lines inside an oscillating magnet array. The success of DNA expression, and vehicle internalisation, were monitored by photometry, fluorescence microscopy, and flow cytometry. Effective magnetofection agents were similar to non-magnetic transfection over long transfection times, however for 1 hour or less of transfection time, magnetofection protocols resulted in approximately 2-fold improved (**) expression at 48 hours post-treatment. The ratio of the rate of vehicle internalisation to the rate of reporter gene expression was found to increase over time (~1 to ~1.2 over 4 hours) and was generally higher in magnetofection (~1.4 vs. ~1.2 for traditional transfection). This leads to the conclusion that magnetofection is not an avenue to universal improvement of transfection outcomes, but has advantages as a technique to shorten the exposure period necessary for effective gene delivery.



# Functional and Structural Studies of a Lipid A Modifying Enzyme in Pathogenic *Neisseria*

Katherine Lim[1], Anandhi Anandan[2], Alice Vrielink[2] & Charlene Kahler [1]

[1]School of Biomedical Sciences, UWA, [2]School of Molecular Sciences, UWA

*Neisseria meningitidis* (Nm) is usually an asymptomatic coloniser of the upper respiratory tract but can cause meningitis and septicaemia in susceptible individuals. *Neisseria gonorrhoeae* (Ng) is a sexually transmitted pathogen that usually infects the urogenital tract. If left untreated in females, it can lead to pelvic inflammatory disease, infertility and spontaneous abortions. Current treatment for gonorrhoea is with antibiotics but widespread global antibiotic resistance has led to the need for alternative treatment methods. The enzyme phosphoethanolamine transferase A (EptA) is conserved in both Ng and Nm, and is a pivotal virulence factor used to transfer phosphoethanolamine from phosphatidylethanolamine to the lipid A of the lipooligosaccharide. EptA is being developed as a therapeutic drug target because inactivation of EptA causes complete attenuation in mouse and human infection models due to its role in protecting the bacteria from cationic antimicrobial peptides, thus enabling the natural immune system to clear the infection. Comparisons between EptA and other members of the alkaline phosphatase superfamily identified several conserved residues in the enzyme active site involved with coordinating the $Zn^{2+}$ ion and substrate binding. These conserved residues were mutated and confirmed to have caused a reduced resistance to polymyxin B. To further understand the impact of these mutations, the mutant proteins were purified and tested for activity and stability. All eight mutant proteins were found to be inactive compared to wild-type (WT) EptA. Biophysical analysis using circular dichroism showed that the secondary structures of all mutant proteins were similar to WT EptA. However, differential scanning fluorimetry results showed three mutant proteins had increased thermal stability compared to WT EptA. Two mutants that had the largest change in thermal stability are undergoing crystallisation trials to examine the effect of the mutations on the binding and active site pocket of the enzyme.

# Investigating the Therapeutic Potential of an Axl Inhibitor, BGB324, in Overcoming Sorafenib Resistance in Hepatocellular Carcinoma

Shelby Margolius[1,2], Tasnuva Kabir[1], Peter Leedman[1]

[1]Laboratory for Cancer Medicine, Centre for Medical Research, Harry Perkins Institute of Medical Research, [2]School of Biomedical Sciences, University of Western Australia

**Introduction:** Hepatocellular carcinoma (HCC) is the 2nd most lethal cancer worldwide, commonly diagnosed at an advanced stage. Currently, sorafenib is the only globally approved primary treatment for advanced HCC, having marginal efficacy as most patients rapidly develop resistance. Recently, we discovered abnormal expression of Axl, a member of the TAM (TYRO3-Axl-MER) family of tyrosine receptor kinases in sorafenib resistance cell lines. Accumulating evidence suggests that overexpression of Axl correlates with poor survival and advanced tumour stage in HCC patients. **Problem statement:** This study investigated the potential of small molecule inhibitor of Axl, BGB324, alone and in combination with sorafenib for treating HCC. **Procedures:** Sorafenib sensitive (Axl naïve) and sorafenib resistant cell lines were treated with sorafenib, and/or BGB324 and cell viability was measured to assess synergy. Inhibition of downstream signalling pathways to Axl and other growth factor receptors were determined using western blot and enzyme-linked immunosorbent assay. The effects on invasion and migration were assessed with 2D transwell assays. **Results:** BGB324 alone inhibited cell viability at low concentrations ($EC_{50}$ = 1.5-2 μM). Results showed a synergistic relationship between sorafenib and BGB324 in sorafenib resistant cells, and promisingly the combination allowed a dose reduction of both drugs. Sorafenib and BGB324 co-ordinately inhibited Axl and downstream signalling through AKT and MAPK pathways and further inhibited activity of the transcription factor c-Jun. Sorafenib treatment alone reduced migration and invasion compared to vehicle control, but BGB324 only affected cell motility in the Axl overexpressing resistant HCC cells. **Conclusions:** In conclusion, BGB324 shows promise for preventing and overcoming sorafenib resistance in HCC where there is currently limited other treatment available to abrogate tumour growth.



# The Influence of the Choice of Force Field on the Characterization of the Monomeric Form of Rat Islet Amyloid Polypeptide (rIAPP)

Sandra Moore, Evelyne Deplazes and Ricardo L. Mancera

School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute and Curtin Institute for Computation, Curtin University

Human islet amyloid polypeptide (hIAPP) is a naturally occurring, intrinsically disordered protein whose abnormal aggregation into amyloid fibrils is a pathological feature in type 2 diabetes. The soluble, oligomeric forms of hIAPP are the most toxic to β-cells in the pancreas. Rat IAPP (rIAPP) differs from hIAPP by 6 amino acids; however, it does not aggregate or form fibrils and, therefore, it can provide a useful comparison to help understand the aggregation process. The structures of the monomeric forms of IAPP are difficult to characterise as they are intrinsically disordered and hIAPP has a tendency to rapidly aggregate into insoluble fibrils. Experimental studies of hIAPP and rIAPP have generally used non-physiological conditions to prevent aggregation but have been unable to describe its soluble monomeric and oligomeric structures under physiological conditions. Molecular dynamics (MD) simulations offer an alternative for the detailed characterisation of the monomeric forms of rIAPP and hIAPP in these conditions. MD simulations, however, can be influenced by the choice of force field and water model used to represent intermolecular interactions. In this work the conformational free energy landscape of rIAPP was predicted as a function of alpha-helical and beta-sheet content using well-tempered metadynamics with walkers. A series of commonly used biomolecular force fields in combination with multiple water models. The predicted conformational preferences of rIAPP are typical of intrinsically disordered peptides, showing both slight alpha-helical and beta-sheet content but with the lowest free energy states reflecting mostly random coil structures. The comparisons of secondary structure content made between different force fields demonstrated the importance of making the right choice when characterising IDPs. Further research will be done on characterising hIAPP and this will be compared with current predictions for rIAPP.

# Simulating the Membrane Binding of the Anti-Cancer Peptide Gomesin

John D. Tanner, Evelyne Deplazes, Ricardo L. Mancera

School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute and Curtin Institute for Computation, Curtin University

Gomesin is an antimicrobial and anticancer peptide originally isolated from tarantula haemocytes. The peptide shows potent cytotoxic activity against clinically-relevant bacteria and fungi as well as a range of human and murine cancers. Gomesin predominately acts by permeabilising cell membranes. Peptides such as Gomesin have therapeutic potential, but a lack of understanding of their molecular mechanisms hinders the rational design of peptides that selectively interact with cancer or microbial cells. Molecular dynamics (MD) simulations can offer a molecular-level insight into the mechanism of these peptide. In this study, we use MD simulations to understand the interaction of Gomesin with model membranes with a view towards predicting the binding affinity and understanding the first steps of membrane permeabilisation. For an accurate and reliable calculation of binding affinity the simulation needs to describe the full range of rotational and translational motion of the peptide on the membrane surface, a task difficult to achieve using conventional MD simulations. To address this, we used a modified version of the enhanced sampling method Replica Exchange with Solute Scaling (REST2), where interactions between the peptide and the surrounding lipid and water molecules are rescaled. We have optimized the scaling factors of the peptide-membrane and peptide-water interactions to increase the sampling of peptide configurations. By comparing conventional MD and REST simulations of the peptide at different distances to the membrane we show that the rotational motion is reduced dramatically as Gomesin approaches the membrane, hindering its ability to sample those high-energy states required for the accurate calculation of the binding affinity (free energy of binding). The methods developed will allow the prediction of the binding affinity of Gomesin and its variants to model cell membranes in order to rationalize its structure-activity relationships.



# The Importance of Mitochondrial Function During Cryopreservation and Recovery

Lily Whelehan[1], Bryn Funnekotter[1,2], Eric Bunn[2], Ricardo L. Mancera[1]

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

Cryopreservation is a relatively new method of plant conservation that allows for much longer storage times than conventional ex situ techniques; However, parts of the cryopreservation process can cause damage to plant tissues. Isolation of plant tissues from the main plant causes mechanical damage. The material can also be physically damaged by the formation of ice crystals during freezing. To alleviate this damage, the material is partially dried prior to freezing, as well as treated with toxic cryoprotectants, both of which cause osmotic stress. All these steps can also cause oxidative damage. Recovery from this damage requires a source of energy, which is supplied by mitochondria. Thus, in order to further improve cryopreservation methods, it is essential to gain an increased understanding of how energy production by the mitochondria is affected by cryopreservation. This project aims to determine whether plant mitochondrial function is damaged by the cryopreservation process. Oxygen consumption of plant shoot tips will be measured as an indicator of mitochondrial function before and after cryopreservation using a Q2 respirometer. We hypothesise that due to the stresses that cryopreservation imposes on mitochondria, oxygen consumption (as a measure of mitochondrial function) will be reduced by the cryopreservation process. We have compared the post-cryopreservation metabolic rate of shoot tips to that of non-frozen, freshly excised shoot tips. As expected, metabolic rate increased in regenerating cryopreserved shoot tips compared to non-regenerating cryopreserved shoot tips.

## Development of a 3D Human Microvessel to Investigate the Mechanism of Sudden Collapse Following Snakebite Envenoming

Sebastian Amos[1,2], Abbie Francis[2,3], Daniel Fatovich[2,3], Erika Bosio[2,3] and Yu Suk Choi[1]

[1] School of Human Sciences, UWA, [2]Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research, [3] Emergency Medicine, School of Medicine, UWA

Introduction. Snake envenomation can cause acute, life-threatening symptoms that require immediate medical intervention. The rapid onset of symptoms such as sudden-collapse makes it almost impossible to investigate the underlying pathological mechanisms of envenoming in a clinical setting. Problem Statement. Given the translational limitations that plague traditional study models, there exists the need to develop a humanised system that accurately emulates the cellular environment of a human microvessel, so that pathologies altering permeability, such as envenomation, may be studied. Procedures. Preliminary investigations will define the physiological parameters of optimal endothelial growth on hydrogel surfaces. This will facilitate the development of a physiologically accurate 3D human microvessel; in contrast to currently published models that fail to incorporate principles of endothelial mechanobiology. Results. Human endothelial cells will vascularise the lumenal surface of 3D hydrogel channels before a microfluidic pump is connected to the device to mimic physiological flow conditions. Changes to vessel permeability and endothelial junction integrity will be assessed using confocal microscopy and other techniques following exposure to snake venom to model sudden-collapse. Conclusions. The aim of this project is to develop a more translational model of distinctly human pathophysiology. Such a physiologically accurate microvessel model would supplement research in many different research areas.



## Secretion of an Extracellular Matrix upon Co-Expression of *Giardia* Cyst Wall Proteins in *Saccharomyces cerevisiae*

Amy Black[1,2], Dr. Carl Mousley[1,2], Dr. Robert Steuart[2]

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

*Giardia duodenalis* has a two-stage lifecycle; the cyst and trophozoite. The environmentally resistant cyst is formed upon encasement of the infective trophozoite within a cyst wall protein (CWP) and carbohydrate matrix by a process called encystation. The three CWPs (CWP1-3) are transported to the cyst wall assembly site via encystation specific vesicles (ESVs). The cyst wall protein complex is believed to form within ESVs however the processes that drive assembly are poorly understood. Few tools are available that allow the *in vivo* molecular dissection of protein complex assembly in *Giardia*. We therefore developed a heterologous system to study the assembly of the cyst wall protein complex in *Saccharomyces cerevisiae*. Cyst wall proteins were cloned into plasmids containing different selectable markers for co-expression and transformed into yeast. Interestingly the co-expression of either CWP1 or CWP3 with CWP2 resulted in cells secreting an 'extracellular matrix-like' material. As both CWP1 and CWP3 only bind to CWP2, it is likely that this matrix is an ESV-like structure that is formed within the cell and secreted as they would in *Giardia*. Interestingly, when colonies were exposed to infrared, only the cells co-expressing CWP1 or CWP3 and CWP2 fluoresced. To determine whether the fluorescence was due to the extracellular matrix, cells were incubated with a reducing agent to break any disulfide bonds. This resulted in a decrease in fluorescence, therefore indicating that the matrix is disulfide bonded CWPs being secreted. Together, we have developed a unique heterologous expression system that will allow us to investigate how the protein component of the *Giardia* cyst wall, which endows environmental resistance, is assembled. In the future, this tool set will help us to obtain a molecular insight into the interactions that are necessary for the assembly of this important structure.

# Characterizing the Structure-function Relationship of the Slc37a2 Transporter

Gerrard Ferreira[1], Jamie Tan[1], Pei Ying Ng[1], Nathan Pavlos[1]

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

Members of the major facilitator superfamily (MFS) of transport proteins are essential for the exchange of a wide range of substrates across membranes of intracellular organelles. Slc37a2 is a member of the SLC37 family of MSF-related sugar-phosphate transporters which consist of 4 structural paralogs: Slc37a1, a2, a3 and a4. Unlike the archetype family member Slc37a4 whose ER-localisation and function in glucose-6-phosphate:Pi exchange is well-characterised, limited information is available regarding the location and physiological function of other SLC37 family members. We have recently identified Slc37a2 as an endolysosomal transporter critical to the bone-resorbing function of osteoclasts. Here using a series of alanine substitution mutations we explored the structure-function relationship of the Slc37a2 transporter. *In silico* structural modelling studies combined with bioinformatics was used to identify key amino acid residues conserved across Slc37a2 from multiple species and within the SLC37 family. Using this approach we identified several amino acid residues predicted to interfer with the substrate binding/Pi-linked antiporter function (R40, K41 and S311) and glycosylation (N53, N62, N68) of Slc37a2. To interrogate the importance of these residues on Slc37a2 subcellular localisation and function we systematically substituted each individual amino acid with alanine (A) and stably expressed each protein as a GFP-fusion chimera in HEK293 cells. Western Blotting and fluorescence microscopy confirmed the expression of the Slc37a2 alanine mutants and their subcellular targeting to endolysosomal membranes. As anticipated mutation of N residues within the extended N-terminal loop of Slc37a2 (Δ3XN) altered the electromobility of the transporter thus confirming the importance of these amino acids for N-linked glycosylation. By comparison, whereas the expression of R40A, K41A and S311A mutants did not alter subcellular targeting of Slc37a2 to endolysosmal membranes, they influenced the size and/or biogenesis of the organelles. Together these preliminary findings provide initial insights into the structure-functional relationship of the Slc37a2 transporter. Future studies will focus on assessing the capacity of these mutants in influence bone-resorption by osteoclasts.



# Investigating the Roles and Regulation of MCAM Ectodomain Shedding in Melanoma

Tenielle George[1], Dr Danielle Dye[1] and Prof Deirdre Coombe[1]

[1]Curtin Health Innovation Research Institute, Curtin University

**Introduction**: Melanoma cell adhesion molecule (MCAM) is a transmembrane protein expressed on melanoma cells, and is linked to tumour progression and poor prognosis. MCAM undergoes a form of post-translational modification, called ectodomain shedding, which gives rise to soluble MCAM (sMCAM). This molecule is functionally relevant in melanoma progression, promoting tumour cell survival and binding to endothelial cells to promote blood vessel formation. Understanding how MCAM is shed from the surface of melanoma cells may aid in the identification of novel clinical markers and therapeutic targets for malignant melanoma. **Problem Statement**. The autocrine and paracrine effects of sMCAM in the tumour microenvironment contribute to melanoma progression, however the mechanisms that regulate MCAM ectodomain shedding in melanoma cells remain largely unknown.**Procedures**. MCAM-expressing melanoma cells were cultured *in vitro* under normal conditions, as well in the presence of phorbol 12-myristate 13-acetate (PMA) to stimulate shedding, or the broad-spectrum metalloproteinase inhibitor, GM6001, to inhibit shedding. The effects of these culture condition on MCAM ectodomain shedding were measured, and cell morphology and/or migration were assessed. **Results**. Constitutive shedding of MCAM occurred in melanoma cells under normal culture conditions. Activation of shedding was stimulated by PMA, which also promoted rearrangement of the cell cytoskeleton and the formation of focal adhesions. Meanwhile, broad-spectrum inhibition of metalloproteinases resulted in impaired MCAM shedding, and appeared to affect melanoma cell migration. **Conclusions**. Melanoma can be aggressive and highly metastatic, and molecules such as MCAM appear to play a role in its metastatic progression. Preliminary data suggests that MCAM undergoes metalloproteinase-dependent shedding, which may enhance cell migration to promote the spread of melanoma. Investigating this further and improving our understanding of how ectodomain shedding of MCAM is regulated, may have implications in the detection and treatment of metastatic melanoma.

# Characterisation of the Endolysosomal Slc37a2 Transporter in Macrophages

Shannon Huntingford, Pei Ying Ng, Katherine Audsley, Jamie Tan, Fiona Pixley and Nathan Pavlos
School of Biomedical Sciences, The University of Western Australia

Endolysosomes are acidic 'hybrid' organelles formed by the transient fusion of late-endosomes and lysosomes. These 'lysosome-related organelles' serve as the major site of hydrolytic enzyme activation and participate in the digestion of cargo acquired during endocytosis, phagocytosis and autophagy. In macrophages, endolysosomes play a critical role in nutrient uptake, microbe killing, antigen processing for presentation and matrix degradation during tumour invasion. To maintain intraluminal acidity and function, endolysosomes are furnished with membrane transporters that control the exchange of ions, amino acids and other solutes between the cytosol and the endolysosomal membranes. Slc37a2 is a member of the SLC37 family of solute carrier transporters thought to function in G6P:Pi exchange. Slc37a2 is expressed in macrophages where it localises to endolysosomes, however, its physiological relevance remains undefined. By combining a series of cell assays with genetically-modified mice lacking the Slc37a2 gene we investigated the role of Slc37a2 in macrophage endolysosomal-related functions. To address whether Slc37a2 influences cargo traffic along the endo-lysosomal network we utilized fluorophore conjugates of endocytic markers to monitor rates of pinocytosis, receptor-mediated endocytosis and receptor-recycling. Slc37a2 knockout and WT splenic macrophages were pulsed with an Alexa Fluor 488 conjugate of dextran, then chased to allow dextran to traffic to and accumulate in endolysosomes. Cells were then pulsed with each Alex Fluor 546 endocytic tracer (0-60 min) and colocalization assessed by confocal microscopy. Slc37a2-knockout cells showed similar cargo internalisation, recycling and degradation kinetics to their WT-counterparts. To examine the effect of Slc37a2 deficiency in microbial activation and killing we assessed macrophage responses to LPS and bacterial phagocytosis (*S.auerus*). LPS induced similar endolysosomal tubulation in Slc37a2 WT and knockout cells, and uptake and formation of phagolysosomes was comparable between Slc37a2 WT and knockout cells. Finally we assessed the capacity of Slc37a2-deficient macrophages to degrade matrix. Preliminary findings indicate degradative capacity of Slc37a2 knockout macrophages is reduced compared to WT cells. From these data we conclude that Slc37a2 does not influence endolysosomal cargo internalisation and trafficking in macrophages but alters their capacity to degrade extracellular matrix possibly owing to impaired delivery and/or secretion of intraluminal enzymes.



# Heterogeneity of Human Dermal Fibroblasts and their Extracellular Matrices

Catherine F LeGrand[1], Chee Wai Wong[1], Deirdre R Coombe[1]
[1]CHIRI, Curtin University, Perth, Australia

**Introduction**. The extracellular matrix (ECM) is important in cutaneous wound healing because it directs the proliferation, migration, and differentiation of multiple cell types. Fibroblasts are the primary producers of ECM in the dermis and recent data revealed dermal fibroblasts are heterogeneous. Dermal fibroblast subpopulations were identified by their differing locations within the dermis, their different morphologies, and their expression of different markers. Evidence from a murine model suggested a specific fibroblast subpopulation is responsible for the production of fibrotic ECMs and for scar formation. We showed that foetal dermal fibroblasts produce an ECM that is compositionally different from an adult dermal fibroblast ECM, an interesting finding given that in the foetus wound healing is regenerative and occurs without scarring. **Problem Statement**. The human dermis contains distinct fibroblast subpopulations, identifiable by different marker expression patterns and these subpopulations generate distinct ECMs. It is likely, the subpopulation proportions differ in the adult and fetal dermis, thereby contributing to the different ECMs. **Results**. Phenotyping of primary foetal and adult human dermal fibroblasts revealed similar proliferation capacities. However, their responses to TGFb-1 stimulation differed, with adult dermal fibroblasts being more responsive as more cells differentiated into myofibroblasts. The fibroblast markers: CD90, CD26, and CD146, were shown by immunofluorescence to be expressed in both foetal and adult fibroblast populations. Flow cytometry revealed these markers were not uniformly expressed. A subpopulation negative for CD90 and CD26 was detected in adult and foetal fibroblasts, while CD146 expression was higher amongst foetal fibroblasts. **Conclusions**. Preliminary data indicates the presence of cell subpopulations within foetal and adult human dermal fibroblasts obtained from two different donors, and it is predicted that the ECM generated from these subpopulations will differ in molecular composition.

# Investigating the Complex Interactions Required ERAD-icate Reduced Sil1p

Thomas McLeod, Robert Steuart, Carl Mousley

Curtin University, CHIRI, School of Pharmacy and Biomedical Science

**Introduction**. Should a newly folded protein fail ER quality control (ERQC) it is targeted for proteasomal degradation through a process known as ER associated degradation (ERAD). The ERAD machinery retro-translocates misfolded proteins across the ER membrane, during which they are concomitantly poly-ubiquitylated, targeting them for degradation. In yeast ERAD is directed by two major E3 ubiquitin ligases, the Hrd1 and Doa10 complexes. The Hrd1 complex consists of many protein subunits with distinct roles in the recognition and retro-translocation of misfolded substrate proteins. In contrast, the Doa10 complex is reported to be much simpler, containing only Doa10p and the E2 enzymes Ubc6p and Ubc7p. **Problem Statement**. It is generally accepted that ERAD is cleanly partitioned with ER lumenal proteins recognized by the Hrd1 complex, and ER associated cytoplasmic proteins recognised by the Doa10 complex. However, a Sil1p variant simulating the Sil1p N-terminus in its fully reduced state ($Sil1^{C52S, C57S}$), that is lumenal, undergoes Doa10p-dependent ERAD. **Procedures**. $Sil1^{C52S, C57S}$ was overexpressed in a panel of mutants defective in either Hrd1 and/or Doa10 dependent ERAD. Yeast harboring either YEp Sil1 or YEp $Sil1^{C52S, C57S}$ were streaked onto –Leu selective medium and incubated at 30°C for 2 days. Exponentially growing WT, *hrd1Δ*, *doa10Δ*, *der1Δ*, and *yos9Δ* cells expressing $Sil1^{C52S, C57S}$ were pretreated for 20 min with 0.25 mg/mL cycloheximide (chx). Cells were removed at 0, 30, 60, and 90 minutes and cell lysates were viewed by immunoblot. **Results**. Expression of $Sil1^{C52S, C57S}$ severely diminished growth of *doa10Δ, der1Δ, and yos9Δ* yeast compared to WT cells. Chx chase was used to further validate these findings. **Conclusions**. Several proteins that are currently believed to only be components of the Hrd1 complex are shared with the Doa10 complex. As ERAD components are highly conserved, our findings provide a blueprint to study the homologous TEB4/MARCH6 complex in mammals.



# Determining the Effects of Low Dose Ultraviolet Radiation on the Circadian Rhythm of Thermogenesis in Brown Adipose Tissue of Mice Fed a High Fat Diet.

Kunjal Panchal[1,2], Naomi Fleury[1], Prue Hart[1], Peter Mark[3], Rachel Crew[3], Robyn Lucas[4], Martin Feelisch[5], Richard Weller[6], Shelley Gorman[2,3]

[1]Murdoch University, [2]Telethon Kids Institute, [3]University of Western Australia, [4]Australian National University, Canberra, [5]University of Southampton, UK, [6]University of Edinburgh, UK.

**Introduction**. Alterations to the body's normal circadian rhythm increase the risk of developing metabolic dysfunction amongst obesity. Thermogenesis in brown adipose tissue (BAT) uses energy stores, and results in the release of heat. Activating thermogenesis in BAT has potential to treat metabolic dysfunction. Thermogenesis in BAT occurs via uncoupled respiration and is closely related to the expression of uncoupling protein-1 (UCP-1), a process with a circadian rhythm. We have shown that ongoing exposure to low dose ultraviolet radiation (UVR) curbed weight gain and limited metabolic dysfunction in mice fed a high fat diet through mechanisms involving skin release of nitric oxide. We hypothesised that regular exposure to low dose UVR may alter the circadian rhythm of thermogenesis in BAT of mice fed a high fat diet, through a nitric oxide-dependent mechanism. **Experimental method**. The circadian rhythm of thermogenesis in BAT was tracked in UCP-1 luciferase transgenic ('thermomouse', FVB/NJ) male mice by injecting mice with luciferin. There were 4 treatment groups in this 12-week study: 1) Mice fed a low-fat diet, and mock-irradiated (n=20); 2) Mice fed a high fat diet, and mock-irradiated (n=20); 3) Mice fed a high fat diet, and exposed twice a week to low dose UVR (1 $kJ/m^2$) (n=20); 4) Mice fed a high fat diet and exposed twice a week to low dose UVR (1 $kJ/m^2$) and topically treated with the nitric oxide scavenger, cPTIO (1 mM) (n=20). At baseline, after 6 and 12 weeks, UCP-1 expression in the interscapular region (major BAT site), dorsal skin temperature and blood glucose were measured quarterly over 28 h. Signs of weight gain, and metabolic dysfunction were also monitored by weighing mice, performing glucose and insulin tolerance tests, and other outcomes. **Conclusions**. This experiment will determine whether changes in thermogenesis are linked the capacity for low dose UVR to suppress metabolic dysfunction in mice fed a high fat diet. Future studies will aim to determine whether exposure to low dose UVR has similar metabolically beneficial effects in humans.

# DDX20-mediated Wnt/β-catenin Signaling Determines Cell Fate Decisions through Cellular Redox in Triple-negative Breast Cancer.

Sebastian Ö-G Pohl[1], S. Pasic[1], J. Suresh[2], R. Carlessi[1], J.L. Hirpara[3], P. Newsholme[1], J. Tirnitz-Parker[1], N. Tolwinski[2], K. N. Keane[1], M. Agostino[1], S. Pervaiz[3], A. Prem Kumar[4], A. Dharmarajan[1]

[1]School of Pharmacy and Biomedical Sciences, Curtin University, [2]Yale-NUS College, [3]Department of Physiology and [4]Cancer Science Institute of Singapore, National University of Singapore.

Reactive oxygen species (ROS) and the evolutionarily conserved Wnt/β-catenin signalling pathway are critical in cell fate, although how canonical Wnt signalling regulates ROS is not fully understood. Aberrant Wnt signalling and oxidative stress are key determinants of triple-negative breast cancer (TNBC) tumourigenesis and drug resistance. Similarly, overexpression of the DEAD-Box helicase DDX20 (Gemin3) in TNBC was directly involved in the acquisition of these traits. Previous studies demonstrated that the regulation of ROS-scavenging enzymes during oxidative stress – which relies on transcriptional activation by β-catenin – is controlled through FOXO transcription factors, or Wnt activation of antioxidant response pathways. We investigated whether DDX20-mediated Wnt signalling is involved in ROS-mediated cell fate decisions. We cultured TNBC cell lines utilising Q-PCR, FACS, Western blotting, Chromatin Immunoprecipitation (ChIP) and Seahorse mitochondrial stress tests to investigate overall changes in cellular redox and mitochondrial function. We validated gene expression using Gemin3 knockout and Gemin3 overexpression *Drosophila* models. We demonstrate that diminished DDX20 results in cell death through mitochondrial dysfunction and increased oxidative stress, primarily through increased hydrogen peroxide ($H_2O_2$). $H_2O_2$ can negatively regulate Wnt/β-catenin signaling through downregulation of β-catenin. We show that DDX20-mediated, Wnt-dependent mitochondrial dysfunction and increased oxidative stress occur through differential transcriptional regulation of ubiquinol-cytochrome c reductase core protein 2 (UQCRC2), superoxide dismutase 2 (SOD2) and catalase by TCF4. Furthermore, our *Drosophila* model demonstrated that regulation of redox regulatory enzymes and mitochondrial copy number changes is a conserved mechanism. We report a new mechanism of canonical Wnt signalling that controls TNBC cell fate through regulation of intracellular redox and mitochondrial function in a DDX20-dependent TCF4-mediated manner. This may be extended to understanding the role of Wnt/β-catenin signaling in other developmental and disease contexts, involving the dysregulation of ROS.



# Identification of an Endolysosomal Membrane Transporter Critical for Osteoclast Function

Amy Ribet[1], Pei Ying Ng[1], Jamie Tan[1]& Nathan Pavlos[1]

[1]Bone Biology and Disease Group, School of Biomedical Sciences, The University of Western Australia,

Solute carrier (SLC) transporters are a superfamily of transmembrane proteins that participate in the transport of ions, amino acids and other solutes across membranes. The importance of SLC transporters in human health is becoming increasingly recognised with many recently linked to the pathogenesis of several human diseases. During a quantitative proteomic screen in osteoclasts, we identified the solute carrier protein 37 family member SLC37a2 as a candidate protein whose expression was robustly up-regulated during osteoclast differentiation. The increase in SLC37a2 protein expression was confirmed by immunoblotting using a peptide antibody raised against the hydrophilic loop of Slc37a2. To address the physiological function of this transporter in osteoclast and bone homeostasis we generated a mouse model lacking *Slc37a2*. Using microCT and histology we show that *Slc37a2*-deficieint mice exhibit a high bone mass (intermediate osteopetrosis). Ex vivo analysis of osteoclasts cultured on bone confirmed that this phenotype is attributable to cell-autonomous defects in osteoclast bone resorptive function. To gain further insight into the function of Slc37a2 in osteoclasts we characterised its subcellular localization using confocal microscopy. We show that Slc37a2 localised to a dynamic network of endolysosomes in osteoclasts. This localisation was confirmed biochemically by endolysosomal enrichment assays and detailed characterisation of endolysosomes isolated from osteoclasts derived from SLC37a2 wildtype and knockout mice. Our results confirm that endogenous SLC37a2 is localized to endolysosomes in osteoclasts where it is co-enriched with established endolysosomal membrane (LAMP2, Rab38) and cargo markers (cathepsin K). Based on these findings we concluded that SLC37a2 represents a previously unappreciated endolysosomal transporter that is critical to osteoclastic bone resorptive function. This research has shed light onto the pathophysiological function of a previously uncharacterised solute carrier protein in bone homeostasis and disease. This may prove helpful in the diagnosis of previously unclassified forms of osteopetrosis.

# Exploring Cell Cycle Checkpoint Inhibition and Gemcitabine Treatment for Glioblastoma

Tracy Seymour[1,2], Mani Kuchibhotla[1], Anna Nowak[2], Nicholas Gottardo[2,3], Raelene Endersby[2]

[1]Telethon Kids Institute, Nedlands, Western Australia, [2]Medical School, University of Western Australia, Crawley, [3]Perth Children's Hospital, Nedlands, Western Australia

**Introduction**. Glioblasotma is the most fatal malignant human brain tumour. Standard treatment for glioblastoma includes safe maximal surgical resection, followed by radiotherapy and adjuvant chemotherapy with temozolomide. The median survival from diagnosis is <15 months and importantly, these treatments are not curative. **Problem Statement**. Glioblastoma cells have the ability to repair treatment-induced DNA damage which contributes to poor patient outcomes. This clearly highlights the need for more effective treatment strategies. **Procedures**. Our study explored gemcitabine combined with LY2606368. Gemcitabine is a small brain-penetrant cytosine analogue that causes DNA damage and initiates the DNA damage response pathway. LY2606368, is an inhibitor of cell cycle checkpoint 1 and 2 (CHK1/2) which enhances the effects of DNA-damaging chemotherapies in other adult cancer. We hypothesise that LY2606368 will inhibit gemcitabine-induced DNA damage repair, leading to DNA damage accumulation and enhanced glioblastoma cell death. Drug interaction assays and western blot analysis determined the compatibility between gemcitabine and LY2606368 in several glioblastoma cell lines. The immediate cellular effects and survival benefit were examined using orthotopic mouse models. **Results**. LY2606368 synergised and improved gemcitabine-induced cell death in two glioblastoma cell lines, T98G and U87. Western blots with patient-derived GBM6 and GBM39 glioblastoma cells showed LY2606368 inhibited CHK1, while combination with gemcitabine reduced phosphorylation of CDC2 and increased gammaH2AX levels. These data suggests that LY2606368 mediates a block in cell cycle arrest, resulting in accumulated DNA damage. Similar results were observed *in vivo*. Immunohistochemistry showed LY2606368 increased gemcitabine-induced DNA damage and reduced tumour cell proliferation. More importantly, using several orthotopic mouse models of glioblastoma, we showed that co-administration of LY2606368 with gemcitabine significantly extended survival. **Conclusions**. This study established the therapeutic potential of co-administrating gemcitabine and LY2606368 and provides robust data that will enable an informed rational for future clinical trials with glioblastoma patients.



# Seeding of the Fetal Microbiome

Lisa F Stinson[1], Matthew S Payne[1], and Jeffrey A Keelan[1]

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

**Introduction**. Early-life microbial colonisation is believed to play a role in immune programming and later life health, yet the evidence regarding the origins, timing and significance of the neonatal microbiome remains inconclusive due to problematic study design, biased amplification and contamination. **Procedures**. Placental, amniotic fluid, first-pass meconium and cord blood samples were collected from 50 elective Caesarean section deliveries. An optimised sampling and analysis protocol was employed to minimise/control for contamination, allowing characterisation of the fetal gut microbiome and its relationship with maternal health parameters, short chain fatty acid (SCFA) levels and fetal immune responses. **Results**. All meconium samples contained detectable levels of bacterial DNA and the immunomodulatory SCFAs acetate and propionate, confirming the hypothesis that the fetal gut is inoculated with bacteria/bacterial DNA in utero. At the phylum level, meconium was dominated by Proteobacteria and Firmicutes. Pelomonas - known to be protective against pathogenic fungal infections - was the most abundant genus. Importantly, this genus has been found in the core non-pregnant endometrial microbiome, but not paired vaginal samples. Lactobacillus (which dominates the vaginal microbiome) was found in only 5 samples. Maternal atopic disease was associated with decreased staphylococcus abundance in the fetal gut. The amniotic fluid microbiome was distinct from the meconium microbiome and was dominated by skin commensals, suggesting early niche construction. **Conclusions**. Seeding of the fetal microbiome commences prenatally and may originate from the endometrial microbiome present at time of conception; vaginal contribution appears minimal. Maternal health may influence fetal immune programming via modulation of the fetal microbiome and immunomodulatory SCFAs. Microbial niche differentiation likely begins prior to birth.

## Method Development for Vaginal Microbiome Analysis in the Context of PTB

Alishum Ali[1,2,3]; Claus Christophersen [1,2]; Matthew Payne[3,4]; Michael Bunce[1,2]; Jeffrey Keelan[1,2,3,4]

[1]Trend Laboratory, Curtin University, [2]Western Australia Human Microbiome Collaboration Centre, [3]Women & Infant Research Laboratory, University of Western Australia, [4]King Edward Memorial Hospital

Introduction. Preterm birth (PTB) is all birth <37 weeks of gestation affecting 15 million pregnancies yearly. PTB aetiology is still unknown, however an imbalance in the vaginal microbiome has been causally implicated. For many years studying the complete microbiome was impossible due to the majority of organisms being uncultivable with traditional methodologies. However; NGS has made it possible to characterise all members present in a particular environment by sequencing short regions of a phylogenetically informative genes such as the 16S rRNA gene. Despite the usefulness of short read sequencing the technology has many challenges that needs to be mitigated. Procedures. Six deidentified mid-vaginal samples were supplied by through the WA Pregnancy Biobank managed by King Edward Memorial Hospital (KEMH). Sequencing was done in paired-end using an Illumina MiSeq500 instrument. Experimentally we compared; (A) 2x DNA extraction methods, (B) bacterial community structure according to V4(300bp) and V6(100bp) regions of the 16S rRNA gene, (C) 2x library preparation protocol and (D) 3x bioinformatic pipelines. Results. V6 region produces higher bacterial richness compared to the V4 region. Preliminary analysis attributes observed difference in bacterial community to well-known primer and PCR amplification biases. The effects of the latter biases seem to remain despite steps taken to increase PCR efficiency and eliminating inhibitors. We further mitigated amplification biases by employing a ligation-based sequence library preparation. Effectively, a ligation method eliminates a second round of amplifications. Study was heavily limited by sample number. Employing a mock bacterial community allowed for tracking of biases post DNA extraction. Our study, reflects the disagreement in the literature surrounding short read sequencing in microbiome studies. Conclusion. We conclude that amplicon sequencing coupled with correct experimental controls is a versatile tool for presence absence studies and may give indication to certain bacterial taxa abundances.



## A Novel Therapeutic Strategy for Marfan Syndrome Utilising Antisense Oligonucleotides.

Jessica M Cale[1, 2], Kane Greer[1, 2], Sue Fletcher[1, 2], Steve Wilton[1, 2].

[1]Centre for Comparative Genomics, Murdoch University; [2] Perron Institute for Neurological and Translational Science

**Introduction**. Marfan syndrome is an autosomal dominant connective-tissue disorder, caused by fibrillin-1 (*FBN1*) mutations, that affects between 2 and 3 in 10,000 individuals. Fibrillin-1, is a large glycoprotein that aggregates into multimer units to form the backbone of microfibrils an essential component of the connective tissue. **Problem Statement**. The pathogenesis of Marfan syndrome is not fully understood, however, *FBN1* mutations are hypothesised to result in monomers that are unable to form multimers, leading to a reduction in fibrillin-1 deposition and subsequent dysregulation of transforming growth factor beta. This study demonstrates a potential therapeutic strategy for a Marfan syndrome patient carrying a *FBN1* mutation resulting in splicing of an exon from affected mRNA transcripts. This strategy relies on the excision of the exon from unaffected mRNA transcripts, re-establishing the homogeneity between fibrillin-1 monomers. We hypothesise that the resulting monomers will form multimers, increasing microfibril formation and reducing disease severity. **Procedures**. Antisense oligonucleotides, designed to target regulatory splicing motifs within *FBN1* exon 52, were screened in unaffected and patient fibroblasts, to assess excision of exon 52 from unaffected transcripts. Treated cells were also immunostained to reveal changes in the abundance and morphology of fibrillin-1 post treatment. **Results**. The observed exon 52 skipping was dose dependant, with up to 96% of transcripts in patient fibroblasts lacking exon 52. A corresponding increase in fibrillin-1 staining and return of fibre-like morphology was observed in treated, compared to untreated patient cells. **Conclusions**. The use of antisense oligonucleotides to induce targeted alternative splicing has garnered attention in recent years, particularly for treatment of Duchenne muscular dystrophy. We believe this technique is applicable to Marfan syndrome, with preliminary in vitro data supporting the hypothesis that inducing homogeneity between fibrillin-1 monomers has therapeutic potential.

# Disease-associated Mutations in *ZBTB18* Disrupt its Neuronal Functions Through a Mechanism Involving Transcriptional Regulation

I.A. Hemming*[1,2], O Clément*[1,2,4], I.E. Gladwyn-Ng[1], H.D. Cullen[1,2,4], H.L Ng[2], E See[1], L Ngo[1], D Ulgiati[2], K.D.G. Pfleger[1,2,3], M Agostino[5,6], J. I-T. Heng[1,2,3,4,6] *Co-first

[1]The Harry Perkins Institute of Medical Research, [2] University of Western Australia, [3]Centre for Medical Research. [4]Curtin Health Innovation Research Institute, [5]Curtin Institute for Computation, [6]Curtin University

During the development of the mammalian cerebral cortex, appropriate numbers of neurons, glial cells, and oligodendrocytes must be generated and functionally integrated to form neuronal circuitry. The regulation of gene expression by DNA-binding transcription factors is crucial for this development, and abnormal brain development can result in intellectual disability. The transcription factor ZBTB18 has been reported to regulate cerebral cortical development by suppressing the transcription of target genes. Human genetic association studies have recognised the importance of ZBTB18 for human neuronal development, with genetic mutations to *ZBTB18* associated with abnormal brain development and intellectual disability in humans. However, the causative nature of genetic mutations to *ZBTB18* remains to be clarified. This study investigates the possible pathological consequences of two individual *de novo,* missense mutations in *ZBTB18* (p.N461S and p.R495G), detected in two unrelated patients diagnosed with intellectual disability. Immunolocalisation studies revealed that the subcellular localisation of the two mutated proteins differs to the wild-type protein. Strikingly, a luciferase reporter assay revealed a unique outcome for the p.R495G mutation, which exhibited transcriptional activation rather than repression. The p.N461S mutation was also observed to disrupt the transcriptional regulatory activity of ZBTB18. Thirdly, *in utero* electroporation experiments show that both missense mutations have different capacities to restore the defective migration of Zbtb18 shRNA-treated cells. Altogether, the findings demonstrate that these disease-associated mutations alter the transcriptional regulatory function of ZBTB18, and impair its capacity to control radial migration during cerebral cortex development. We conclude that the presence of N461S or R495G missense mutations compromises sequence-specific DNA-binding by ZBTB18, and impairs its transcriptional regulation and radial migration functions *in vivo*. The approach undertaken can provide a blueprint for systematically interrogating the mechanisms underlying disrupted transcriptional regulation of neuronal positioning genes concerning associated brain disorder.



# Therapeutic Potential of Antisense Oligonucleotide-mediated Exon Inclusion for Stargardt Disease

Di Huang[1,2,3], Sue Fletcher[1,3], May T Aung-Htut[1,3], Norman Palmer[3], Fred Chen[2,4], Steve Wilton[1,3]

[1]Centre for Comparative Genomics, Murdoch University ; [2]Centre for Ophthalmology and Visual Science & Lions Eye Institute, The University of Western Australia; [3]Perron Institute for Neurological and Translational Science; [4]Department of Ophthalmology, Royal Perth Hospital.

**Introduction**. Stargardt disease (STGD1) is an autosomal recessive juvenile-onset macular dystrophy caused by mutations in the ATP-binding cassette transporter gene (*ABCA4*). ABCA4 is localized to the rims of the outer segment disc membranes of photoreceptors and plays a crucial role in the removal of excessive 11-*cis* and all-*trans* retinal. Mutations in ABCA4 can lead to progressive accumulation of cytotoxic bisretinoid compounds and eventually to the death of photoreceptors and retinal pigment epithelium cells. The precise pathogenic mechanisms of most mutations are not known and there is no effective treatment. **Problem Statement**. The c.5461-10T>C mutation in the *ABCA4* transcript was reported to compromise normal pre-mRNA processing, resulting in mature mRNA transcripts missing exon 39, and exons 39 and 40. Deletion of either or both exons causes a frameshift in the *ABCA4* transcript, most likely resulting in nonsense mediated degradation (NMD). Splice modulating antisense oligonucleotides (AOs) have been shown to promote exon inclusion in other conditions and we hypothesize that the same strategy may be applicable to STGD1 patients carrying the c.5461-10T>C mutation. **Procedures**. We designed several AO sequences that target intronic splicing silencers within intron 39 to enhance inclusion of exon 39 or exon 39 and 40 in the mature *ABCA4* transcript. The AOs were transfected into patient fibroblasts carrying the c.5461-10T>C mutation and the *ABCA4* transcript was analyzed after 24 hours. **Results**. AO intervention mediated an increase in the full-length *ABCA4* transcript with a concomitant decrease in the transcript missing exon 39 in treated, compared to the untreated fibroblasts. These observations confirmed that NMD of the *ABCA4* transcript was prevented by exon 39 inclusion. Further studies to assess ABCA4 protein expression and function after treatment are in progress. **Conclusions**. This study demonstrates that a splice modulating AO has therapeutic potential for STGD1 patients carrying the c.5461-10T>C mutation through splice correction.

# Case study: Duchenne Muscular Dystrophy and Autism Arising from a Complex X-Chromosome Rearrangement in a Male Patient

Niall Keegan[1,2,3], Steve Wilton[1,2,3,4], Stacey Tay[5], Swati Tomar[5], Raman Sethi[5], Abbie Adams[1,2,3], Russell Johnsen[1,2,3], Lai Poh San[5], Abha Chopra[1,6], Sue Fletcher[1,2,3,4]

[1]Murdoch University, [2]Perron Institute, [3]Centre for Comparative Genomics, [4]UWA, [5]National University Hospital Singapore, [6]Institute for Infectious Diseases

The male patient was diagnosed with severe autism spectrum disorder (ASD) and Duchenne muscular dystrophy (DMD) at a young age. In accordance with classical DMD pathology, the patient had elevated serum creatine kinase levels, and western blots performed on his muscle biopsy extract revealed an absence of detectable dystrophin protein. However, various genomic analyses of the patient's *DMD* gene, including whole genome and whole exome sequencing (WGS and WES), indicated that all *DMD* exons and their flanking intronic regions were intact. RT-PCRs of the patient's *DMD* mRNA detected no abnormal splicing, although the quantity of transcript appeared to be extremely low. Believing that the patient may possess a deep intronic mutation that induced intron inclusion, we performed whole transcriptome sequencing (WTS) of total RNA extracted from his muscle biopsy and aligned the resulting reads to a 5' segment of the *DMD* gene. This alignment revealed substantial inclusion of introns 1, 2 and 9, with virtually no aligned reads 3' of intron 9. Manual interrogation of the aligned reads eventually discovered a fusion of *DMD* intron 9 with *GLRA2* intron 8. Subsequent re-inspection of WGS data confirmed this mutation at the genomic level, as well as discovering two others involving *CFAP47*. The interlocking nature of these three gene fusions indicated that they constitute a single rearrangement of the patient's X-chromosome, with no duplications and a minimal loss of intron sequence. Because prior research has linked mutations of *GLRA2* and *CFAP47* with autism, we therefore conclude that this complex genomic rearrangement explains both the patient's DMD and his ASD. Our findings highlight the difficulty some genetic disease patients and their families still face in obtaining accurate diagnoses, despite the rapid advance of sequencing technologies.



# Measuring the Impact of Genetic Knowledge on Intentions and Attitudes of the Community Towards Expanded Preconception Carrier Screening

Royston Ong[1,2], Denise Howting[1,2], Alethea Rea[3], Hayley Christian[4], Pauline Charman[5], Caron Molster[6], Gianina Ravenscroft[1,2], Nigel George Laing[1,2]

[1] Centre for Medical Research, UWA, [2] Harry Perkins Institute of Medical Research, [3] Centre for Applied Statistics, UWA, [4] School of Population and Global Health, and Telethon Kids Institute, [5] BioDiscovery Centre, Harry Perkins Institute for Medical Research, [6] Department of Health Western Australia

Preconception carrier screening (PCS) provides the potential to empower couples to make reproductive choices before having an affected child. An important question is what factors influence the decision to utilise or not utilise preconception carrier screening. As there is no existing PCS program publicly available currently, we set out to answer six questions: *Will they use PCS*; *How much are they willing to pay*; *Who they want to access PCS from*; *What diseases do they want screened*; *Is there a correlation between genetic knowledge, attitudes and intentions; are there other possible factors*? We analysed the correlation between genetic knowledge, attitudes and intentions to participate in PCS using logistic regression in 832 participants in Western Australia**.** Two-thirds of participants said they would take the test, with 92% of these supporting screening for diseases reducing the lifespan of children and infants. Those who had good genetic knowledge were seven times more likely to intend to use PCS (p=<0.001), while those with high genetic knowledge were four times more likely to (p=0.002) and raised concerns such as insurance and confidentiality. Decreasing genetic knowledge correlated positively with religiosity and apprehension (p=<0.001), which correlated negatively with intention to use PCS (p=<0.001). Increasing genetic knowledge correlated positively with factors representing positive attitudes (p=<0.001), which correlated positively with intention to use PCS (p=<0.001). Many participants with good genetic knowledge nevertheless answered questions that tested understanding incorrectly. 80% of participants stated they would prefer to access the test through their general practitioners and 30% would pay up to AUD200. We conclude that knowledge is instrumental in influencing participation. Having good genetic knowledge may not be enough to understand core concepts of PCS and may impact informed decision-making. This study recommends that continuous education of health professionals and thus the community, in PCS is crucial to reduce misconceptions.

# Investigating Polymorphic Intronic Variants as Potential Biomarkers for MND

Julia Pytte[1,2]; Ryan S. Anderton[1,2]; Loren Price[1,3]; Anthony Akkari[1,3]

[1]Perron Institute for Neurological and Translational Sciences,. [2]School of Health Sciences and Institute for Health Research, the University of Notre Dame. [3]Centre for Comparative Genomics, Murdoch University

Motor neuron disease (MND) is a progressive neurodegenerative disease that results in the loss of neurons within the central nervous system, leading to muscle paralysis and eventual death. In 90% of cases, MND occurs with no prior family history, and despite the advancement of Genome Wide Association Studies, only 11% of the total sporadic cases have identifiable gene mutations. Non-coding polymorphic structural variants are understudied biomarkers that can enrich clinical trials and lead to potential therapeutic targets for MND. Therefore, the aim of this study was to investigate the presence of polymorphic variants within, and in close proximity to, associated MND genes. Potential influential genetic variants associated with MND genes were identified using a short structural variant evaluation algorithm. A database of these variants was created and prioritised according to their potential impact on MND genes. Each variant was systematically assessed for polymorphisms through polymerase chain reaction, polyacrylamide gel fractionation and Sanger sequencing. Several variants currently reported as monomorphic in the NCBI database and literature around MND genes, such as *VCP*, *FUS*, *SQSTM1*, and, *SOD1*, have been found as polymorphic in our assessments. Such polymorphic variants may have the ability to regulate and alter mRNA transcription and gene expression of key MND-associated genes. The identification of high impact polymorphic variants is the first step towards developing biomarkers for MND. To confirm our current results, future studies will investigate these newly identified polymorphic variants in both healthy individuals and patients with MND. The results of such an association study may assist in identifying target genetic sequences for the screening and determination of MND.



# Developing Personalised Molecular Therapy for Vascular Ehler Danlos Syndrome Using Splice Modulating Antisense Oligonucleotides

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

[1]Faculty of Medicine Siriraj hospital, Mahidol University, Thailand, [2]Centre for Comparative Genomics, Murdoch University, [3]Perron Institute for Neurological and Translational Science

**Introductions.** Vascular Ehler Danlos Syndrome (vEDS) is a connective tissue disorder caused by mutations in *COL3A1* encoding collagen III. Collagen III is composed of three COL3A1 polypeptide monomers coiled around each other as a homotrimers that are secreted into the extracellular matrix (ECM) and form collagen fibril. Collagen III is mostly present in the ECM of internal organs such as lungs, uterus and vascular wall. Therefore, defects in collagen III compromise internal organs. Three types of *COL3A1* mutations; missense, nonsense and splice site, have been reported and affect production, secretion and thermal stability of collagen III. Antisense oligonucleotide (AO) mediated splice modulation to alter exon selection is showing potential for the treatment of several diseases. **Problem Statement.** We propose that a personalised medicine using splicing modulating AOs could be beneficial for vEDS patients. **Procedures and Results.** The patient's mutation (IVS14-2A>G) in the *COL3A1* was confirmed by isolating and sequencing DNA and RNA from patient-derived fibroblasts. Analysis of the *COL3A1* transcript from patient fibroblasts with the splice site mutation, IVS14-2A>G, showed exon 15 skipping, and immunostaining of collagen III showed intracellular accumulation. Since the patient fibroblasts express two transcript variants, with and without exon 15, different collagen III proteins are produced and hence impair proper assembly and secretion of the collagen III trimer. Our strategy is to induce skipping of exon 15 from the transcript encoded by the unaffected allele and correct the collagen periodicity and rescue trimer assembly. AOs with 2'-O-methyl modified bases on a phosphorothioate backbone, were designed to skip *COL3A1* exon 15. AOs were transfected into the patient's fibroblasts and splice modulation efficiency was assessed by RT-PCR amplification of the *COL3A1* transcript. The optimal AO sequences will be sourced as phosphorodiamidate morpholino oligomers, a chemistry that has been validated for clinical use, and functional analysis will be performed.

## Neutrophils Increase in Lymphoid Tissues and Tumours in Elderly Tumour-Bearing Mice

Karlo Aguda[1], Pat Metharom[1], Connie Jackaman[1]

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

**Introduction**. Ageing is associated with impaired immune function which can lead to deteriorating health and increased cancer prevalence, such as mesothelioma. Anti-tumour immunity involves the recognition and elimination of tumour cells by cytotoxic T cells (CTLs), however, T cell function is impaired in ageing. Recent studies have shown that key innate immune cells, neutrophils can regulate T cell activation in lymph nodes via antigen presentation or through mediating other key immune cells such as dendritic cells and macrophages. **Problem statement.** Neutrophils can polarise into either anti-tumour N1 or pro-tumour N2 neutrophils. N2 neutrophils have the ability to suppress CTLs and promote tumour growth. However, few studies have examined age-related changes to neutrophils within tumours and their potential contribution to declining T cell function in ageing. **Procedures**. Female C57BL/6J mice were inoculated with AE17 murine mesothelioma. Bone marrow, spleen, lymph node and tumours were collected from mice aged 3-4 months (corresponding to 18 human years) and 22-24 months (corresponding to 60-70 human years). Immunofluorescence examined the location of neutrophils ($Ly6G^+$), relative to blood vessels (CD31) and $CD8^+$ T cells from cryosections. Flow cytometry was used to identify/sort for neutrophils ($F4/80^{neg}SSC^{hi}CD11b^+Ly6G^+$). Nuclear morphology was assessed to confirm for neutrophils based on nuclei segmentation. **Results**. $Ly6G^+$ cells infiltrated into spleen, lymph nodes and tumours from blood vessels, and were more prevalent in elderly healthy and tumour-bearing mice compared to young. Nuclei of cells sorted from tumours had characteristics similar to segmented neutrophils, with majority exhibiting hypersegmented (more than 5 lobes) morphology and were increased in tumours from elderly mice. **Conclusions**. This study demonstrates that neutrophils infiltrate into the elderly tissue microenvironment where they have the potential to interact and influence T cells. Ongoing studies are examining both the age-related neutrophil function and whether their interaction with T cells is altered during ageing.



## Identification of Latent Epstein-Barr Virus in B-cell Subsets using Confocal Microscopy

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 and Infectious Disease, [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 is the causative agent of infectious mononucleosis and is implicated in several human oncogenic diseases as well as autoimmune diseases including multiple sclerosis. Latent infection can be identified through Epstein-Barr Nuclear Antigen-1 (EBNA-1) and Epstein-Barr virus-encoded small RNAs (EBER) which are constitutively expressed in latency. Although it is known that EBV infects human B-cells, it is unknown which B-cell sub-population EBV primarily resides in. **Problem statement.** The study aims to visualize EBV in B-cell subsets using confocal microscopy and SmartFlare™ technology, targeting EBNA-1 and EBER via complementary RNA coupled flourescent nanoparticles. **Procedures**. martFlares were tested using healthy human controls and EBV-transformed cell-lines derived from these. Healthy controls were serologically tested for EBV positivity using ELISA technology and EBV copy number was quantified using the QIAGEN EBNA-1 qPCR kit. For confocal microscopy, peripheral blood mononuclear cells of healthy controls were B-cell enriched by depleting $CD4^+$ and $CD8^+$ T-cells using magnetic beads. Cells were incubated with SmartFlares for 16hrs, subsequently washed, fixed to coverslips, and incubated with fluorescent antibodies targeting CD19, CD20, IgG and CD27 to distinguish subsets using the Nikon $C2^+$ Confocal microscope. **Results**. The results of the study show that EBNA-1 and EBER SmartFlares successfully bind to their targets. Both, EBV transformed cell-lines and healthy controls showed EBV residing in memory B-cells. Additionally, due to its high expression, EBER positive cells were more frequently detected than EBNA-1 positive B-cells. **Conclusions**. Future studies will aim to confirm presence of EBV in memory B-cells by Flow-sorting individual B-cell subsets and subsequently testing these subsets with the previously optimized EBNA-1 qPCR. By elucidating which primary B-cell subset serves as the viral reservoir it is possible to develop targeted monoclonal antibody treatments to deplete specific virally infected cells with minimal side effects.

# Effect of Pre-adapted HIV on Disease Outcome Following Vertical Transmission

Jennifer Currenti[1], Abha Chopra[2], Shay Leary[2], Elizabeth McKinnon[2], Mina John[2,3], Spyros Kalams[4], Mark Pilkinton[4], Simon Mallal[2,4], Silvana Gaudieri[1,2,4]

[1]School of Human Sciences, UWA, [2]Institute for Immunology and Infectious Diseases, Murdoch University, [3]Department of Clinical Immunology, Royal Perth Hospital [4]Division of Infectious Diseases, Vanderbilt University, Nashville, TN

Human immunodeficiency virus (HIV) is responsible for more deaths than any other infectious disease worldwide. Currently, there is no cure for HIV infection with the only option being lifelong antiretroviral treatment. HIV adapts to an individual's anti-HIV immune response over time via mutations in its genome, allowing it to undermine the immune response and negatively influence disease outcome. Furthermore, these adaptations can be transmitted. To design an effective preventative and/or therapeutic vaccine, it is vital to understand the complex host-viral interactions: namely how transmission of pre-adapted HIV affects disease outcome in the recipient. Previously, the level of adaptation of the transmitted virus has been correlated with disease outcomes in horizontal transmission cases. This study examined the effect of transmission of pre-adapted HIV on clinical measures of disease outcome following vertical transmission. HIV clade B sequences were determined for 26 mother-child transmission pairs using next-generation sequencing. The resultant sequences and previously determined viral adaptations were used to assess adaptations present and calculate adaptation scores using in house bioinformatic tools. The transmitted virus was highly adapted to the child's anti-HIV immune response and showed limited reversion. Furthermore, there is evidence to suggest a possible transmission network from father-to-mother-to-child. A significant correlation was observed between mothers' adaptation scores and viral load ($p<0.05$); however, this was not reflected in the child. These results suggest that adaptations present in the mother are transmitted to the child, having the potential to impact their disease outcome. However, the lack of correlation between the level of adaptation and clinical measures of disease outcome support the hypothesis that host-viral interactions in children are not the same as adults, likely due to infection with a highly pre-adapted virus. Understanding the interactions between a highly adapted virus and host is a fundamental aspect of vaccine design.



# Flavivirus-specific Cellular Immune Responses in Travellers with Previous Dengue and Zika Virus Infection.

Hannah Greig[1], Suzi McCarthy [2,1], Laila Abudulai[1], Allison Imrie[1], David Smith[2]

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

**Introduction**. Zika virus (ZIKV) recently caused outbreaks associated with severe neurological symptoms. ZIKV is phylogenetically and structurally similar to dengue virus (DENV), a flavivirus which exerts a major burden of disease in equatorial regions. ZIKV and DENV pose diagnostic challenges as they cross react, complicating accurate diagnosis of past infections. Measurement of virus-specific immunological memory is an important marker of past infection and is used in vaccination trials as one indicator of a successful vaccine. ZIKV and DENV have no licenced vaccines and a correlate of immune protection has yet to be established. Accumulating evidence suggests that humoral memory may not indicate establishment of protective memory and that the role of the cellular immune response is vital in clearing infection. **Problem Statement**. Currently, cellular immunity is measured by HLA typing a cohort, synthesizing peptides for probable immunogenic epitopes and using these peptide antigens to measure cytokine response in PBMC. This is often not feasible for large cohort studies where sample size is limited and may not represent the preferred epitopes used in *in vivo* antigen presentation. **Procedures**. To circumvent using peptides, whole virus was used to stimulate interferon gamma release by flavivirus-specific lymphocytes. These were quantified by enzyme linked immunospot forming assay (IFNγ ELISpot). Responses were measured in 9 patients with prior ZIKV or DENV infection. **Results**. 24h was the optimum period of stimulation for measuring flavivirus-specific IFNγ release. 8/9 patients matched serological data of prior flavivirus vaccination. 2/5 patients with past DENV showed an INFγ response against DENV-2. No patients showed responses to ZIKV. **Conclusions**. Whole virus stimulation can be used to detect flavivirus-specific cellular memory in vaccination or infection in some patients. Further optimisation or investigation of other memory cells is required to use whole virus stimulation as a measure of cellular immunity.

# Meningococcal Serogroup Y Disease in the Senior Population of Western Australia

D. He, C. M. Kahler, and S. Mowlaboccus

The Marshall Center for Infectious Diseases Research and Training, School of Biomedical Sciences, UWA.

**Introduction**. *Neisseria meningitidis* (meningococcus) expressing capsule serogroup A, B, C, W, X or Y causes invasive meningococcal disease (IMD). Multi-locus sequence typing classifies meningococci into genetic lineages termed clonal complexes (cc). Since 2015, Western Australia (WA) has seen a significant increase in meningococcal serogroup Y (MenY), especially in the senior population. Furthermore, an increase in penicillin-resistant meningococci has also been observed. **Problem Statement**. The WA-MenY collection was assessed for penicillin resistance and characterised by whole-genome sequencing to investigate the association of genetic lineage with age. **Procedures**. A significant proportion of MenY disease was observed in the senior population of Australia post-2012. In WA, 19 culture-confirmed MenY cases were reported from 2005-2017. To characterise these, whole-genome sequencing was performed using Illumina paired ends. The raw reads were assembled using Velvet Assembler, auto-tagged and curated using the BIGSdb genomics platform available on the PubMLST database. **Results**. MenY incidence was observed in all age groups but was significantly higher in patients aged above 55 years ($p<0.05$). MenY isolates belonged to cc23 (n=17) and cc167 (n=2). Eight different sequence types (STs) were identified – ST-23 (n=7), ST-6800 (n=4), ST-1655 (n=3), ST-884 (n=1), ST-3582 (n=1), ST-4183 (n=1), ST-6799 (n=1) and ST-11432 (n=1). Core-genome phylogenetic analysis identified two clusters within cc23. Cluster 23.1 contained 10 penicillin-susceptible isolates (MIC=0.064 mg/L). Cluster 23.2 contained 7 isolates with intermediate levels of resistance to penicillin (0.125 mg/L). An examination of variant loci of penicillin binding proteins known to be involved in conferring penicillin resistance were identical in all isolates. This may suggest a novel mechanism for penicillin resistance among cluster 23.2 isolates. **Conclusions**. MenY was identified as the major cause of IMD in the senior population of WA. Core-genome phylogeny identified a MenY:cc23 cluster that was less susceptible to penicillin but this phenotype should not affect clinical treatment of this disease.



# Pro-Inflammatory Macrophages Impair Skeletal Muscle Regeneration in Elderly Mice

Bridgette Lee[1], Federica Tomay[1,2], Lelinh Duong[1,2], Danielle E. Dye[1,2], Hannah Radley-Crabb[1,2], Connie Jackaman[1,2].

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

**Introduction**. Although skeletal muscle has excellent regenerative capacity, elderly persons experience delayed skeletal muscle regeneration following injury compared to young people. Macrophages play a crucial role in muscle regeneration, and central to this is their ability to transition between pro and anti-inflammatory phenotypes. **Problem Statement**. Macrophages play an important role in muscle regeneration in the young, however it is unknown how macrophages affect elderly muscle regeneration. **Procedures**. C57BL/6J mice aged 3 and 24 months (equivalent to 18 and 65-70 human years, respectively) were injured with notexin in tibialis anterior muscles to induce injury. Muscles were collected on days 1, 2, 4, 7, 10, 14 after injury. Mice underwent macrophage inhibition (α-F480 antibody) on day -2 to day 4 during injury (early inhibition; EI) or day 4 to 10 post-injury (late inhibition; LI). Histology assessed necrosis and central nuclei. Plasma chemokines were measured using bead array. **Results**. Elderly repair was delayed and associated with increased necrosis which declined more slowly than in young. Similarly, central nuclei (indicating regeneration) persisted in elderly up to day 14, whereas they declined at day 10 in young. Central nuclei was not significantly changed in young by EI or LI relative to controls at day 10. However, EI decreased central nuclei in the elderly, and LI increased central nuclei relative to controls at day 10. In the elderly, systemic pro-inflammatory chemokines (monokine induced by γ-interferon; interferon-γ induced protein 10) were increased, which was reduced with EI. **Conclusions**. H&E and chemokine analysis shows macrophages play a key role in skeletal muscle regeneration in young mice. However, during ageing, macrophages display dysregulated inflammatory response post injury which delays repair. Early inhibition of pro-inflammatory macrophages accelerates repair following injury in the elderly. Further studies are ongoing to assess the therapeutic potential for targeting these cells in the elderly.

# The Role of Chronic Hyperinsulinemia in Dendritic Cell Metabolism and Function

Wei Khei Lim[1], Philip Newsholme[1], Delia Nelson[1], Connie Jackman[1], Rodrigo Carlessi[1]

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

Low grade chronic inflammation is a hallmark of chronic diseases of ageing, such as obesity and type 2 diabetes. Patients with these diseases are often found to present with abnormally high circulating insulin levels (hyperinsulinemia). Emerging evidence suggests that hyperinsulinemia could drive diet-induced obesity. It is also known that macrophages in adipose tissue of obese people secrete pro-inflammatory cytokines which can inhibit insulin responsiveness. This indicates that metabolic pathways and innate immune responses are tightly interconnected. We hypothesise that chronic hyperinsulinemia could reshape the immune system, particularly dendritic cells (DCs), leading to chronic inflammation. Bone marrow cells from C57BL/6J mice were differentiated into DCs *in vitro* in the absence or presence of 100 nM insulin. Then, DCs were exposed to LPS for different lengths of time. Various metabolic and functional parameters were analysed, including glycolytic and mitochondrial metabolism by extracellular flux analysis, glucose consumption and uptake rates, intracellular glycogen stores, as well as cytokine secretion. Preliminary work showed that DCs grown in insulin have increased metabolic rates as evidenced by enhanced extracellular acidification rates (ECAR) and oxygen consumption rates (OCR). DCs differentiated in the presence of insulin also presented a more pronounced response to LPS. This was evidenced by significantly higher secretion of the cytokines TNF, IL-6 and Interferon-γ, in addition to an increased ECAR response to acute LPS exposure. These data show that hyperinsulinemia can modulate DCs metabolism and function towards a phenotype characterized by increased responsiveness to LPS. Further work will investigate the role of hyperinsulinemia in other aspects of DC biology such as surface markers expression, antigen presentation capacity and ability to stimulate T cell proliferation. This study will increase our understanding of the intricate relationship between metabolic signals and the regulation of the immune system, and provide novel targets for the treatment of chronic inflammatory diseases.



# Characterisation of the Macrophage Immune Response to *Burkholderia pseudomallei* Infection in the Presence of Novel Inhibitors

Dion Lin-Sullivan[1], Ulrike Holzgrabe[2] Mitali Sarkar-Tyson[1], Jua Iwasaki[1]

[1]Marshall Centre for Infectious Diseases, UWA [2]Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Germany

**Introduction**. Melioidosis is an infectious disease endemic to tropical and sub-tropical areas of the world with foci in Northern Australia and South-East Asia. Treatment for this disease is lengthy and requires significant use of antibiotics. Novel inhibitors have been developed that target a bacterial virulence protein known as the macrophage infectivity potentiator (Mip) and can effectively 'disarm' the bacteria and limit bacterial-attributed cytotoxicity. **Problem Statement.** Currently little is known about the immune response to *B. pseduomallei* infection in the cell line used to test these Mip inhibitors (J774 murine macrophage), and as yet no work has been done to characterise any immunomodulation that may occur by use of these inhibitors and thereby allow the clearance of infection. **Procedures**. An *in vitro* infection model was used with *B. pseduomallei* K96243 in the presence and absence of the Mip inhibitor, SF354 (50μM). Rapamycin, a commercially available drug known to inhibit Mip was used as a control. Supernatants were collected at 0, 1, 4, and 24 hours and assessed for intracellular counts by colony forming units, cell death by lactate dehydrogenase release, and production of 9 pro- and anti-inflammatory cytokines as determined by Luminex™ Multiplex technology. **Results**. SF354 did not demonstrate a significant difference in cell-only cytotoxicity or in intracellular counts from that of DMSO (solvent control). Rapamycin, however, had notable cytotoxicity by 24 hours. SF354 and rapamycin resulted in decreased production of MCP-1, IL-1β, and IL-6 post-infection (as well as TNF-α for rapamycin), when compared to DMSO. **Conclusions**. Decreased production of IL-1, IL-6, and TNF-α in *B. pseudomallei* infection indicates that severity of infection in J774 macrophages is limited by the use of Mip inhibitor SF354. These cytokines are noted in the literature to be important in sepsis – as such, SF354 should be investigated further in *in* vivo studies and pre-clinical trials.

# Illuminating the Immune Microenvironment in Group 3 Medulloblastoma using Multicolour Immunofluorescence

Emily Tan[1], Courtney George[2,3], Mathew Ancliffe[1,3,4], Tamara Abel[3], Raelene Endersby[3].

[1]University of Western Australia, [2]Edith Cowan University, [3]Telethon Kids Institute, [4]Murdoch University

**Introduction**. Medulloblastoma is the most common malignant paediatric brain tumour. Of all the subgroups, Group 3 medulloblastoma has a dismal prognosis with survival rates <40%, despite aggressive multimodal treatment regimes. In recent years, immunotherapy has emerged as a promising adjunct to conventional therapy, yet functional characteristics of the immune microenvironment and activation of specific immune populations in medulloblastoma remain unknown. Guided by preliminary flow cytometric data, we aimed to explore the function of myeloid cells in a Group 3 mouse model of medulloblastoma using multicolour immunofluorescence microscopy. **Procedures.** To visualise the location of specific myeloid cell populations in medulloblastoma, Tyramide Signal Amplification (TSA) was used. This enzyme-based technique, combined with multiple fluorescence substrates, enables simultaneous visualisation of multiple proteins even if the primary antibodies are derived from the same species. Staining and image acquisition parameters were optimised using brain tissue from medulloblastoma-bearing immune-competent mice. **Results.** Preliminary stains demonstrated abundant cells in mouse brain that were positive for TMEM119, a microglial-specific marker. Microglia are the resident macrophages of brain, and while these cells were distributed throughout the normal parenchyma and tumour periphery, they did not infiltrate the tumour. In contrast, Iba-1 positive cells, which marks peripheral macrophages as well as activated microglia, were seen in both the normal parenchyma and within medulloblastomas. **Conclusion.** Our findings suggest that medulloblastoma-infiltrating myeloid cells may not solely be derived from the tissue resident microglial pool. Instead, haematogenous recruitment of peripheral monocytes or macrophages may play a more important role in the immune microenvironment of medulloblastoma. Future work will evaluate the changes to immune cell infiltration following conventional cytotoxic therapy. This will provide insight to the viability of current immunotherapies in this disease, and guide the application of novel therapeutic agents to improve treatment outcomes in medulloblastoma.



# Characterising the Role of the Immune System in the Therapeutic Response to Cyclophosphamide Chemotherapy in Cancer

Caitlin M. Tilsed[1,2], Rasa Islam[1,2], Vanessa S. Fear[1,2], Anna K. Nowak[1,3], Scott A. Fisher[1,2], Richard A. Lake[1,2], W. Joost Lesterhuis[1,2]

[1]National Centre for Asbestos Related Diseases, [2]School of Biomedical Science, [2] School of Medicine, The University of Western Australia, Perth, Australia

Chemotherapy is the first-line treatment for many advanced cancers, yet it is usually not curative. Some cancers do not respond to chemotherapy and patients with similar cancers respond differently. This spectrum of response is also observed in murine models in which genetically identical mice bearing monoclonal tumours have a differential response to chemotherapy. What separates responders and non-responders is not fully understood. Specifically, it is not known how in the tumour microenvironment modulates chemotherapy efficacy. In this study, we investigated the role of the immune system in cyclophosphamide-mediated cures in murine models. Immunodeficient NSG and wild type BALB/c mice were subcutaneously inoculated with CT26 colon cancer and AB1-HA mesothelioma and treated with the maximum tolerated dose of cyclophosphamide (270mg/kg IP single dose). Tumour growth and survival were monitored. In separate experiments, wildtype tumour-bearing mice received cyclophosphamide combined with antibodies depleting T/B/NK cells. Flow cytometry was used to characterise tumour-infiltrating leucocytes associated with a complete or a partial response. No tumours were cured by cyclophosphamide in NSG mice however 60% and 80% were cured in wildtype mice bearing CT26 and AB1-HA, respectively. When $CD8^+$ T cells were depleted in wildtype CT26 tumours, complete responses were completely abrogated, while CD4 and NK cell depletion showed intermediate effects. Preliminary flow cytometry data showed a difference in pre-treatment tumour cellular composition between mice that demonstrate a partial or a complete response. These results highlight that the adaptive immune system is required for a complete response to cyclophosphamide and is predominantly $CD8^+$ T cell dependent, and that the pre-treatment microenvironment may play a role in determining future response. Understanding the requirements for a successful response to chemotherapy would allow development of predictive biomarkers and highlight pathways that could be targeted to improve treatment efficacy.

## Acid Saline Lakes as a Source of New Biomining Organisms.

Katelyn Boase[1], Elizabeth Watkin[1], John Moreau[2], Himel Khaleque[3]
[1]Curtin University, [2]Melbourne University, [3]CSIRO Land and Water

Biomining is a growing industry aimed at providing an environmentally friendly, sustainable and economically viable process, to extract valuable metals from low grade ores. Acidophilic microorganisms that oxidise iron and sulphur are currently being used in this process. These microbes however are sensitive to chloride ions as chloride can pass their cellular membranes and disrupt their reversed membrane potential, causing the acidification of their cytoplasm. Fresh water is therefore required in biomining processes however, this challenges the economic feasibility of biomining in countries where fresh water is scarce. Thus, this study aims to explore acid salt lakes, in search for iron and sulphur oxidising acidophiles that are chloride resistant. Lake Tyrrell, an acid saline lake in north western Victoria, harbours the niche environment these microbes would most likely be present, as it has low pH (~4), high salinity (>200 g.L-1 NaCl) and large iron and sulphur deposits. Water, sediment and biofilm samples were collected and the diversity of the microbial populations were determined using culture and non-culture dependant methods. Enrichment cultures were grown in Basal Salts media at both 35g/L and 105g/L NaCl and at the given pH the sample site was when taken (ranging from pH 4-5). Acid and $Cl^-$ resistant iron/sulphur oxidising microbes were isolated on ferrous iron overlay plates. From all samples, growth at both NaCl concentrations were observed. Iron oxidation however was only visible in enrichment cultures from a biofilm sample at both NaCl levels. Single colonies were isolated from the same enrichment culture which also displayed iron oxidation on ferrous iron overlay plates at 35g/L NaCl and a pH of 4. No iron oxidation was observed from enrichments generated from sediment samples. This study has resulted in the isolation of an acidophilic iron oxidising microbe that may have an application in the biomining industry.



## Efflux Pump Inhibition in *Klebsiella pneumoniae*

Helen Burge
The University of Western Australia

**Problem:** Antimicrobial resistant (AMR) bacteria are spreading globally at a rapid rate. The global spread of AMR has been fuelled in recent decades by the widespread misuse of antibiotics by both clinicians and agriculture. Resistant bacteria are present in the wider community and are increasingly recognised in drug resistant sepsis. Bacterial efflux pumps make an important contribution to AMR in Gram negative bacteria, such as *Klebsiella pneumoniae.* Efflux pumps are capable of extruding antibiotics from the bacterial cell, where it is most effective. **Solution:** Theoretically, if efflux pumps can be inhibited, this would restore the efficacy of antibiotics that are prone to resistance. To test the efficacy of several potential efflux pump inhibitor (EPI) candidates, namely chlorpromazine, verapamil and pantoprazole and using two reference strains (700603, BAA1705) and two clinical isolates (K1, K14) of *K. pneumoniae,* broth microdilutions were performed to determine the MIC. Serial two-fold dilutions of three antibiotics (meropenem, gentamicin and ceftriaxone) were performed, with and without an EPI, to determine if the EPI changed the MIC. **Results:** the change in MIC with an EPI was greater for meropenem and ceftriaxone than for gentamicin, suggesting that gentamicin resistance in *K. pneumoniae* is either less dependent on efflux pump potentiation, or depends on a different efflux pump family. By contrast, there was a demonstrable change in susceptibility to meropenem and gentamicin with addition of chlorpromazine, a potential efflux pump inhibitor. In the case of verapamil, exposure caused an increase in the MIC of ceftriaxone, suggesting that the bacteria either used verapamil as a substrate, or increased their efflux activity. **Conclusion:** Restoring the efficacy of resistance-prone antibiotics by prescribing an EPI as an adjuvant to antibiotic therapy will broaden the therapeutic options available to clinicians when treating a patient with a resistant bacterial infection and lead to improved patient outcomes.

# DNA-Binding Specificity Controlling Mobilisation of Antibiotic-Resistance Plasmids in *Staphylococcus aureus*

Jade E. Crow[1], Karina Yui Eto[1,2], Charles S. Bond[2] and Joshua P. Ramsay[1]

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

Plasmids lacking genes for horizontal transfer can 'catch a ride' with conjugative plasmids to other cells, in a process known as conjugative mobilisation. The recently characterised pWBG749 family of conjugative plasmids mobilise non-conjugative antibiotic-resistance plasmids by recognising specific *oriT* sites. These *oriT*s have been detected on over 50% of all *Staphylococcus aureus* plasmids, often with up to 3 copies per plasmid. Five distinct versions of the *oriT* sequence and five distinct relatives of pWBG749-family conjugative plasmids have been identified. The *Staphylococcus* Mobilisation Protein O (SmpO) is a DNA-binding protein encoded adjacent to each *oriT* on pWBG749-family conjugative plasmids. Five unique SmpO homologues have been identified and we hypothesise SmpO is a specificity factor for *oriT* recognition and plasmid mobilisation. Four SmpO proteins were successfully purified using nickel-affinity and size-exclusion chromatography. DNA-binding and DNA-footprinting assays were carried out using Surface Plasmon Resonance (SPR). Size-exclusion chromatography and small-angle X-ray scattering experiments revealed $SmpO_{49}$, $SmpO_{45,}$ $SmpO_{408}$ and $SmpO_{Sep}$ each formed tetramers in solution. DNA-binding sites for each *oriT* were identified for $SmpO_{49}$, $SmpO_{45}$ and $SmpO_{408}$ using SPR. Two conserved binding regions, named IR2 and IR2*, were identified on each *oriT*. Binding of the predicted IR2* region was additionally confirmed for $SmpO_{Sep}$. Each IR2* was distinct for each *oriT* and was bound specifically by the corresponding SmpO protein. Critical nucleotides required for SmpO binding were further defined through mutational analysis of each minimal IR2*. The pWBG749 family has a broad capacity for gene transfer in *S. aureus*. However, mobilisation has only been demonstrated for three of the five *oriT* types. This work provides molecular evidence that each *oriT* site is indeed recognised by each pWBG749-family plasmid variant and explains how *oriT* sites are distinguished from each other by SmpO proteins.



# Redefining Antibiotic Resistant Plasmid Transfer in *Staphylococcus aureus*

Amy Davis[1], Riley J.T. Murphy[1], Neville Firth[2] and Joshua P. Ramsay[1]

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

Despite abundant evidence for horizontal transfer of antibiotic-resistance plasmids in *S. aureus*, only 5% of clinically-isolated *Staphylococcus aureus* carry conjugative plasmids capable of transferring themselves between strains. Small antibiotic-resistance plasmids may exploit conjugative plasmids for their own transfer, but seemingly, not all plasmids carry the appropriate genes to do so. Conjugative relaxases are an essential component of the conjugation mechanism, which nick DNA and recruit it to the type-IV secretion system for transfer to the recipient cell. Replication initiator proteins (Rep) - required for plasmid replication - are functionally analogous to conjugative relaxases at a molecular level and likely share a common ancestor. Recently, a Rep protein was demonstrated to enable plasmid mobilisation in *Bacillus subtilis*. In this work, we cloned three distinct Rep genes from *S. aureus* plasmids and tested for their ability to facilitate mobilisation by different types of conjugative plasmids. Each *rep* was cloned into the non-mobilisable plasmid pSK5632 and each resulting clone was introduced into the laboratory *S. aureus* strain RN4220. The distinct conjugative plasmids pSK41 and pWBG4 were introduced into each resulting strain. pSK41 mobilised pSK5632 carrying cloned copies of *repU*, *repL* and *rep2* at rates of $4.0 \times 10^{-7}$, $2.9 \times 10^{-8}$ and $2.2 \times 10^{-8}$ exconjugants per donor, respectively, but did not mobilise the pSK5632 vector alone. pWBG4 did not mobilise any plasmids. Prior to this work only ~25% of non-conjugative *S. aureus* plasmids were predicted to encode conjugative relaxases. However, we now present evidence that three out of the four known rolling-circle-replication initiator proteins may act as conjugative relaxases to facilitate mobilisation by pSK41. Future work will involve characterisation of protein-protein interactions involved in recruitment of the RCR Rep proteins to the conjugative type-IV secretion system.

# Macrophage Infectivity Potentiator Proteins as Novel Anti-Virulence Targets in *Neisseria meningitidis*

Emily Kibble[1,2], Jua Iwasaki[1], Ulrike Holzgrabe[3], Geoff Coombs[2], Charlene Kahler[1], Mitali Sarkar-Tyson[1]

[1] The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Science, UWA, [2] School of Veterinary and Life Science, Murdoch University, [3] Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Germany

**Introduction**. *Neisseria meningitidis* is the bacterial causative agent of invasive meningococcal disease (IMD). The rate of IMD in Australia is increasing, with over 300 cases in 2017. Macrophage infectivity potentiator (Mip) proteins are found in a wide range of pathogens, and are known to be important in survival of bacteria within host cells. Mip proteins represent potential broad spectrum anti-virulence drug targets due to their conserved enzymatic and drug binding domains across species. Novel inhibitors designed to target Mip from other bacterial species have been shown reduce the ability of *N. meningitidis* to infect and survive in host cells. While most bacteria are known to have one Mip protein, *N. meningitidis* encodes for two putative Mip-like proteins. **Problem Statement**. It is hypothesised that Mip proteins and those proteins with high homology to Mip are important novel anti-virulence targets in *N. meningitidis*. **Procedures**. Three insertional deletion mutants have been created in *N. meningitidis*; two single mutants, each lacking one of the two putative Mip proteins, and one double mutant, lacking both of the two putative Mip proteins. These mutant strains have been assessed for survival within host cells. Recombinant *N. meningitidis* Mip protein has been expressed and purified. Novel inhibitors against Mip have been screened for inhibition of enzymatic activity of purified Mip protein, and activity *in vitro*. **Results**. Deletion of the putative Mip proteins has resulted in significantly decreased survival of *N. meningitidis* within macrophages. Novel inhibitors have been shown to have an inhibitory effect on the enzymatic activity of purified recombinant Mip, and result in a significant decrease of bacterial association with host cells. **Conclusions**. Mip and Mip-like proteins have been shown to have a role in cell infection, and represent an important set of anti-virulence targets in *N. meningitidis*.



# Development and Optimisation of U937 Macrophages for Zika Virus Infection and Screening of Novel Antivirals

Melissa Koh Yu Ting[1], Jua Iwasaki[1], Kritu Panta[1] Allison Imrie[1], Mitali Sarkar-Tyson[1]

[1]Marshall Centre for Infectious Diseases, School of Biomedical Sciences, UWA

**Introduction**. The recent Zika Virus (ZIKV) outbreaks with clusters of microcephaly and Guillain-Barré syndrome in the Pacific and the Americas has exemplified the urgent need for antivirals against emerging and re-emerging arboviruses. Macrophages have recently been implicated in ZIKV infection, acting as permissive hosts to allow ZIKV to cross into immune privileged sites, including the central nervous system, leading to exacerbation of the disease (Tang et al., 2016). **Problem Statement.** Macrophage infection models have not been well established to screen ZIKV antivirals. U937 cells are a human myeloid cell line, and we are investigating their potential as a model for ZIKV infection studies. **Procedures**. U937 cells were exposed to 100ng/mL of phorbol myristate acetate (PMA) for 72 hours to allow differentiation to a macrophage-like population and adherence to the plate surface. Mycophenolic acid (MPA), was diluted to 100µM, 50µM, 10µM and 1µM in DMSO and incubated with a known concentration of ZIKVMR766. A focus-forming assay (FFA) employing immunostaining was used to quantify virus replication. The reduction in focus forming units (FFU) in the presence of antivirals was compared to an untreated virus control to obtain a percentage change in viral infectivity. Lactate dehydrogenase (LDH) release was used to monitor cytotoxicity of the drugs against DMSO as the vehicle. **Results**. 100 µM and 50 µM of MPA showed 72.0% and 35.5% reduction in FFU respectively against the virus-only control. There was no significant difference in cytotoxicity by MPA compared to the DMSO control. **Conclusions**. MPA inhibits the ability of ZIKV to form viral plaques in U937 cells in a dose-dependent manner, which is consistent with published findings using Vero cells. We, therefore, propose that a U937 macrophage model is an effective tool for screening novel antivirals, with the potential for broad application among globally emerging arboviruses.

Reference: Tang, H., Hammack, C., Ogden, Sarah C., Wen, Z., Qian, X., Li, Y., . . . Ming, G.-l. (2016). Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. *Cell Stem Cell, 18*(5), 587-590. doi:10.1016/j.stem.2016.02.016

# Inversion of the *css/ctr* Cassette in the Capsule Biosynthesis Island *of Neisseria meningitidis* is not Mediated by RecA

E.C. Mikucki, S. Mowlaboccus, and C. M. Kahler

The Marshall Center for Infectious Diseases Research and Training, School of Biomedical Sciences, UWA.

**Introduction**. *Neisseria meningitidis* expressing capsule serogroups A, B, C, W, X or Y causes invasive meningococcal disease which has a mortality rate of 6%. Capsule synthesis (*cps*) and transport proteins are encoded by genes found within the 25 kb *cps* genetic island. The *cps* island contains two cassettes encoding the synthesis of the polymer (*cssA/B/C/D*) and the genes encoding the ABC transporter (*ctrA/B/C/D*) flanked by two conserved repeat regions termed Region D and Region D'. Region D encodes an operon of four genes, one of which is an active galactose epimerase, while Region D' is an inactivated inverted duplication. Previous studies has revealed that Region D and D' undergo reciprocal recombination that result in the inversion of the *css/ctr* cassette relative to the flanking genome. Inversion of *css/ctr* cassette has been observed in meningococci expressing serogroup B and C capsules, but not assessed in other serogroups. The role of inversion of the *css/ctr* cassette in pathogenesis is unknown. **Problem Statement**. To understand the process of *css/ctr* cassette inversion, the role of the recombinase A (RecA) protein necessary for homologous recombination was assessed for its involvement in this event and in clinical isolates of MenW from WA. **Procedures**. *A recA::aphA-3* mutagenic cassette was constructed and introduced in *N. meningitidis* strain NMB (serogroup B). *Css/ctr* cassette inversion in RecA mutant, the parent strain NMB and the clinical MenW isolates was assessed via PCR. **Results**. *Css/ctr* cassette inversion was observed in all of the clinical MenW isolates. Both strain NMB and its isogenic *recA::aphA-3* mutant retained the ability to invert the *css/ctr* cassette.**Conclusions**. In conclusion, *css/ctr* cassette inversion occurs in clinical MenW isolates, and is not RecA dependent. Recombination can be facilitated by other recombinases in Neisseria and these proteins will be assessed for involvement in *css/ctr* cassette inversion using a similar strategy.



# Introduction of Sequence Type 612 Methicillin Resistant *Staphylococcus aureus* into Western Australian Hospitals

Riley JT Murphy[1], Geoffrey W. Coombs[2] and Joshua P Ramsay[1]

[1]Curtin Health Innovation Research Institute and School of Pharmacy and Biomedical Sciences, Curtin University, Western Australia, [2]School of Veterinary and Laboratory Sciences, Murdoch University, Western Australia

*Staphylococcus aureus* is an opportunistic pathogen of humans and animals. Community-associated methicillin resistant *S. aureus* (MRSA) infections are becoming increasingly problematic globally. Sequence type 612 MRSA is dominant in South African hospitals, but is otherwise uncommon. In Australia, ST612 MRSA was detected in a screen of Australian equine veterinarians and was the most prevalent sequence type in an outbreak of MRSA in horses from a New South Wales veterinary clinic. The clinical incidence of ST612 in Western Australia is low overall, but has been steadily increasing over the past 5 years, culminating in a bloodstream infection in 2016. Given the rising incidence of ST612 infection and the dominant status of ST612 in South Africa, we sought to determine the origins of this uncommon sequence type in Western Australian hospitals. Isolates were gathered from the 2008 outbreak in horses in New South Wales and from Western Australian and South African hospitals. Isolates were subjected to antibiotic susceptibility testing, whole genome sequencing, multi locus sequence typing, virulence and resistance gene characterization and phylogenetic analysis. A core genome SNP maximum-likelihood phylogenetic tree was constructed, which clearly distinguished isolates of South African clinical and NSW horse origin into two major clades, with Western Australian clinical and screening isolates clustering closely to isolates in both clades. Combined phylogenetic and molecular data indicates multiple transmissions from both horses to humans and from South Africa into Western Australian hospitals may have occurred. Minor variation in virulence-gene content between all ST612 isolates suggest that ST612 can infect horses and humans without undergoing major adaptive evolution. The high prevalence of rifampicin and trimethoprim resistance in ST612 isolates suggests that the use of these antibiotics for human and horse health may have contributed to the introduction of ST612 into Australia, highlighting the risks of using essential human antibiotics in animals.

# Impact of Host and Microbial Factors on Mesothelial Cell Responses in Peritoneal Dialysis Associated Peritonitis

Hui Juin Shak[1], Kieran Mulroney[1], Nicholas Topley[1,3] & Aron Chakera[1,2]

[1]Translational Renal Research Group, Harry Perkins Institute of Medical Research, QEII Medical Centre, [2]Renal Unit, Sir Charles Gairdner Hospital, [3]Wales Kidney Research Unit, Division of Infection and Immunity, School of Medicine, Cardiff University.

**Introduction**. Peritonitis is one of the major complications of peritoneal dialysis (PD) and is strongly associated with mortality and is most commonly caused by Gram-positive bacteria. The initial phase of the host response to bacteria-causing peritonitis influences long-term outcomes and is mediated by mesothelial cells that line the surface of the peritoneal cavity. **Problem Statement**. In this study, we aimed to examine bacterial and host factors that contribute to the activation of mesothelial cells during peritonitis caused by *Staphylococcus aureus;* one of the most common causes of PD-peritonitis. **Procedures**. Clinical *S. aureus* isolates (n=8) collected from patients with peritonitis were grown in different used PD effluents (n= 4) for 4 hours. Bacterial counts were determined hourly using the spot-plating method to assess inter-strain growth characteristics and the influence of different dialysates on growth. To assess mesothelial cell activation by bacteria, isolates of heat-killed *S. aureus* (n=8), were co-incubated with the mesothelial cell line – MeT-5A for 4 hours in Dulbecco's Modified Eagle's Medium, or used dialysate samples. Supernatants were collected and concentrations of IL-8, a validated marker of mesothelial cell activation were determined by enzyme-linked immunosorbent assay (ELISA). **Results**. Growth curves for different *S. aureus* isolates were equivalent in used PD effluents. There was significant variability in mesothelial cell activation by heat-killed S. *aureus* isolates with no impact of bacterial density on results in concentrations ranging from $10^2$-$10^7$ cfu/mL. Used dialysate samples from patients without peritonitis strongly activated mesothelial cells compared to heat-killed *S. aureus* and unused PDE ($P<0.05$). **Conclusions**. Our study provides the first comprehensive data on the growth of different *S. aureus* strains in dialysis effluents and the impact of heat-killed *S. aureus* and used dialysate to activate mesothelial cells. Our results suggest a dominant role for dialysis solutions in peritoneal inflammation.



# Use of Flow Cytometry to Rapidly Determine the Antimicrobial Susceptibility of Fastidious Bacteria Responsible for Meningitis and Gonorrhoea

Carmon R. Williams[1], Christine F. Carson[1], Shakeel Mowlaboccus[1,2], Teagan F. Paton[1,3], Kieran T. Mulroney[4,5], Nadia Urosevic[1], Timothy J. J. Inglis[1,3,51]

School of Biomedical Sciences, UWA, [2] Marshall Centre, School of Biomedical Sciences, UWA, [3] PathWest Laboratory Medicine, QEII Medical Centre, [4] Harry Perkins Institute of Medical Research, [5] School of Medicine, UWA

**Introduction:** Antimicrobial resistance is a major public health threat that comes at a severe mortality, morbidity and economic cost. Current antimicrobial susceptibility test (AST) methods take over 36 hours to produce a result and are too slow to guide early treatment decisions. This results in poor patient outcomes and increased resistance selection pressure. The flow cytometry-assisted susceptibility test (FAST) produces results in less than 3 hours. To date, this technique has only been used with easily grown bacteria. **Problem Statement:** The fastidious bacteria that cause infections such as gonorrhoea and bacterial meningitis also require rapid AST results. The primary objective of this study is therefore to adapt the FAST method for use with fastidious organisms. We aim to develop FAST for common causes of bacterial meningitis: *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae,* as well as the causative agent of gonorrhoea, *N. gonorrhoeae*. **Procedures:** In this study, representative isolates from each species were grown in liquid culture and exposed to a series of concentrations of ceftriaxone and benzyl penicillin. After antibiotic exposure, bacterial cells were stained with a fluorescent nucleic acid binding dye. An acoustic flow cytometer was then used to capture fluorescence and light scatter data from antibiotic-unexposed and exposed bacteria. **Results:** With these data we obtained the MIC, the minimum antibiotic concentration at which an inhibitory effect was demonstrated. These results were then compared to MIC values obtained by conventional AST methods. **Conclusions:** We have shown that FAST can be adapted to fastidious bacteria, determining MIC results in less than 6 hours. This represents a substantial improvement on current AST methods. Further development and implementation of the FAST method will significantly improve patient outcomes by providing AST results to drive earlier, evidence-based treatment decisions.

# Serum Biomarkers Associated with Non-motor Symptoms in People with Parkinson's Disease

Megan Bakeberg[1-2], Maddeson Riley[1], Michelle Byrnes[1-2], Frank L. Mastaglia[1-2] and Ryan S. Anderton[1-3*]

[1]Perron Institute for Neurological and Translational Science, [2]Centre for Neuromuscular and Neurological Disorders, UWA, [3]Institute for Health Research, University of Notre Dame Australia

**Background:** Parkinson's disease is considered a multidimensional disorder which can frequently result in a range of non-motor symptoms, such as cognitive dysfunction and impulsive behaviour. Demographic variables and dopamine D2 agonist medication can predispose people with Parkinson's disease (PwP) to be more impulsive. However, it is unknown which PwP are more susceptible to greater trait impulsivity. **Objective:** Given the importance of iron and copper regulation in dopamine homeostasis within the brain, this study aimed to investigate serum ferritin and ceruloplasmin as predictors of impulsivity in PwP. **Methods:** Serum iron studies and ceruloplasmin levels were obtained from 214 PwP enrolled in the Australian Parkinson's Disease Registry, and 78 aged-matched controls. Barratt Impulsiveness Scale (BIS-11) measures patient impulsivity, and were obtained from patients in the ON state. Multivariate general linear models (GLMs), controlling for demographic and medication variables, were used to determine whether serum markers associated with impulsivity. **Results**: No differences were observable in serum ferritin, transferrin or ceruloplasmin between PwP and controls. Only serum ceruloplasmin was significantly associated with total BIS-11 scores, whether a dichotomised (20 g/L) or continuous variable. GLMs controlled for confounding variables, leaving ceruloplasmin and gender as factors that significantly predict higher BIS-11 total scores, second order non-planning domain, and first order subscales. **Conclusions:** Elevated serum ceruloplasmin levels are associated with heightened trait impulsivity, specifically nonplanning impulsivity in PwP. Therefore, serum ceruloplasmin may serve as a specific marker for impulsivity in PD patients, and aid in the identification of individuals more susceptible to harmful behaviours such as pathological gambling.



# Novel Nucleic Acid Technologies to Tackle Alzheimer's Disease

Madhuri Chakravarthy[1,2] & Rakesh N. Veedu[1,2]

[1]Centre for Comparative Genomics, Murdoch University, Australia, [2]Perron Institute for Neurological and Translational Science, Australia.

**Introduction**. Nucleic acid technologies are a validated therapeutic strategy for various diseases. Nusinersen, an antisense oligonucleotide drug that was approved by the US FDA for the treatment of spinal muscular dystrophy highlights the potential of nucleic acid technologies in the treatment of other neurological diseases. **Problem Statement**. We have explored the potential of novel nucleic acid technologies in treatment of Alzheimer's disease. **Procedures**. We designed a range of antisense oligonucleotide candidates across various exons of the BACE1 gene and tested the efficiency of the synthetic antisense oligonucleotide candidates to inhibit the BACE1 transcript in vitro through splice modulation. **Results**. Preliminary results have identified an antisense oligonucleotide that can effectively reduce BACE1 mRNA expression by 84% and will need to be further validated. **Conclusions**. This study has developed nucleic acid technologies that may have therapeutic potential for Alzheimer's disease.

F3

# Systematic Evaluation of 2'-Deoxy-2'-Fluoro (2'-F) Modified Antisense Oligonucleotide-mediated Exon Skipping for Duchenne Muscular Dystrophy

Suxiang Chen[1,2], Bao T. Le[1,2], Madhuri Chakravarthy[1,2], Rakesh N. Veedu[1,2]

[1]Centre for Comparative Genomics, Murdoch University, [2]Perron Institute for Neurological and Translational Science

**Introduction**. Chemically modified antisense oligonucleotide (AO)-mediated exon skipping has been established as a successful strategy for the treatment of Duchenne muscular dystrophy (DMD). Phosphorodiamidate morpholino (PMO) and 2'-O-methyl phosphorothioate (2'-OMe-PS) chemistries were used in clinical research to develop exon-skipping AOs for tackling DMD. 2'-Deoxy-2'-fluoro (2'-F) is an attractive analogue that shows enhanced nuclease stability and affinity to DNA or RNA targets and can be explored for their applicability in exon skipping. **Problem Statement**. PMO oligos showed excellent safety profile, however, PMO is not compatible with other chemistries to synthesize chimeric AOs to further improve the efficacy and in addition, large scale production of PMOs is challenging due to difficult synthesis chemistry which make it expensive. 2'-OMe-PS is compatible with other chemistries but has safety issues and shows limited efficacy. **Procedures**. To overcome these, we envisioned the development of novel chimeric AOs (gapmer and mixmer) using 2'-F-PS nucleotide. In our study, we explored the scope of a 2'-OMe/2'-F-PS gapmer (AO1) and two mixmers (AO2, AO3) together with a locked nucleic acid (LNA)/2'-F-PS gapmer (AO4) and two mixmers (AO5, AO6) to induce exon-23 skipping in *mdx* myotubes *in vitro*. We then conducted cell viability assays and nuclease stability assays in order to assess the cytotoxicity and stability profiles of these chimeric AOs. **Results**. All of the 2'-OMe/2'-F-PS mixmers (AO2, AO3) and LNA/2'-F-PS chimeras (AO4, AO5, AO6) induced higher exon-23 skipping (51%, 46%, 54%, 47%, 53%) compared to their fully 2'-OMe-PS AO control (45%) while only one 2'-OMe/2'-F-PS mixmer, AO1 induced less skipping (26%) at 12.5 nanomolar concentration. In addition, all chimeric AOs did not show any significant cytotoxicity, and they showed high stability to exonuclease degradation. **Conclusions**. Based on our preliminary results, 2'-F modified chimeric AOs could be useful in achieving efficient splice modulation in cells

F4

# Poly-Arginine Peptides Improve Functional Recovery and Reduce Neuroinflammation Following Traumatic Brain Injury

Li Shan Chiu[1,2], Ryan Anderton[1,2,4,5], Vince Clark[1,2,3], Jane Cross[1,2,3], Neville Knuckey[1,2,3], Bruno Meloni[1,2,3]

[1]Perron Institute for Neurological and Translational Science, [2]Centre for Neuromuscular and Neurological Disorders, UWA, [3]Department of Neurosurgery, Sir Charles Gairdner Hospital, [4]School of Heath Sciences, UNDA, [5]Institute for Health Research, UNDA

**Introduction**. Cationic arginine-rich peptides (CARPs) such as the poly-arginine R18 and R18D peptides have demonstrated neuroprotective efficacy in *in vitro* neuronal excitotoxic injury and *in vivo* rat models of stroke. These peptides have also demonstrated significantly reduced axonal injury and positive trends for functional recovery in a rat model of traumatic brain injury (TBI). **Problem Statement**. Currently, there are no neuroprotective pharmacological treatments for TBI. Since poly-arginine peptides have demonstrated neuroprotective potential, both R18 and R18D were further characterised in a rat model of TBI. **Procedures**. Using a weight-drop impact-acceleration apparatus, a closed-head TBI was induced in male rats. A dose-response study using R18D (100, 300, 1000 nmol/kg) administered intravenously at 30-min post-injury was conducted in Sprague-Dawley rats. A second study in Long-Evans rats examined R18 and R18D at a dose of 1000 nmol/kg administered intravenously at 30-min post-injury. **Results**. In the Sprague-Dawley rat, doses of R18D at 100 and 1000 nmol/kg significantly reduced the extent of axonal injury in the corpus callosum, and demonstrated significant improvement in learning and memory outcome. In the Long-Evans rat, both R18 and R18D significantly reduced astrocytic activation, and reduced IL-6 levels in the brain following injury. R18D was particularly effective at improving sensorimotor function and generally produced more favourable functional outcomes than R18. **Conclusions**. Poly-arginine peptides have neuroprotective effects following TBI and warrant continued investigation as a novel therapeutic for TBI.

F5

# Assessing the Effect of Intestinal Permeability on Neuronal-like cells: An Enteric Model to Study Parkinson's Disease.

Christi Gildenhuys [1,2], Anastazja Gorecki [1], Gabriella MacDougall[1,2], Bruno Meloni [1,3], Gerard Hoyne [2], Ryan S. Anderton [1,2].

[1]Perron Institute for Neurological and Translational Science, [2]Institute for Health Research, University of Notre Dame
[3] Department of Neurosurgery, Sir Charles Gairdner Hospital

**Introduction:** Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, with a vast majority of cases (~90%) remaining idiopathic. The low penetrance of PD, relevance of environmental contributors and complex pathogenesis has led to a "multiple hit theory" as the causative sequence. This suggests several insults of disease-specific risk factors are required for the characteristic dopaminergic cell loss within PD. Although this hypothesis supports a multifactorial disease progression, little is known about the specific mechanism by which these contributors cause neurodegeneration. **Objective:** The present study focuses on developing and characterising an *in vitro* enteric model of PD that incorporates two leading risk factors of PD: an alpha-synuclein protein aggregation and an altered microbiome. **Procedures:** To replicate an altered microbiome, rat small intestinal (IEC-6) cells were treated with lipopolysaccharides (LPS) and assessed by immunohistochemical and Western blot analysis for tight junction protein expression. Neuroblastoma (SH-SY5Y) cells expressing alpha-synuclein were established as a model of enteric neuronal-like cells, and subjected to LPS (0.1 – 20 g/ml) and oxidative stress insults (20 – 100M ). Cell viability was determined by LDH release, propidium iodide staining and Western blot analysis of caspase-3. **Results:** Following LPS stimulation, expression of ZO-1, claudin-1 and occludin were altered in IEC-6 cells, resulting in a potentially compromised intestinal epithelial barrier (IEB). Mimicking a more permeable IEB, SY-SY5Y cells expressing mutant alpha-synuclein showed significantly greater cell death following either $H_2O_2$ or LPS treatments. **Conclusion:** Development of this enteric model of PD provides an *in vitro* tool that successfully integrates both alpha-synuclein expression and an altered IEB. This model could potentially aid further characterisation of the causative mechanism behind PD and initial trialing of future therapeutics.

F6

# Low Intensity rTMS Improves Cognition in a Mouse Model of Alzheimer's Disease: Frequency Dependent Effects

Alesha Heath[123], Rachel Sherrard[1]

[1]Biological Adaptation and Ageing, UPMC, Sorbonne Universités, Paris, France, [2]Experimental and Regenerative Neuroscience, School of Biological Sciences, UWA, Perth, Australia, [3]Perron Institute for Neurological and Translational Science, Perth, Australia

Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising technique to target cognitive decline in Alzheimer's disease (AD), although there is some concern that improved AD scores after treatment reflect alleviated depression rather than actual cognitive improvement. While high intensity rTMS is the main focus of current research, low intensity stimulation delivered diffusely to the whole brain has also proved beneficial. When delivered focally, low intensity rTMS (LI-rTMS) has been shown to affect intracellular calcium, BDNF and neuronal branching, indicating this form of magnetic stimulation may be appropriate for targeting specific aspects of neuroplasticity and therefore help improve cognition. We treated six month PS1M146V female mice, a genetic knock in model of AD, and C57BL6/J age-matched controls with LI-rTMS (12mT) focused over the hippocampus. We tested the effects of four different frequencies, two classically excitatory: BHFS and 10Hz and two classically inhibitory: 1Hz and cTBS. After two weeks of stimulation we found positive effects of the two excitatory frequencies in restoring Morris Water Maze spatial memory deficits and dendritic spine abnormalities. Whereas the two inhibitory frequencies had different effects, with 1Hz stimulation inducing partial improvements and cTBS having either no effect or in some cases worsening these measures. However, none of the stimulation protocols improved the loss of neurogenesis levels in AD mice. Importantly, the stimulations had no effect on the anxious phenotype seen in the Elevated Plus Maze, suggesting that the behavioural effect was solely due to an improvement in cognition. These results suggest excitatory LI-rTMS applied to the hippocampus could provide a targeted treatment of particular synaptic deficits underlying cognitive impairment in Alzheimer's Disease.

F7

# Trait Impulsivity in Parkinson's Disease: Patient Quality of Life Implications

Ashani Jeyadevan[1,2], Michelle Byrnes[2], Souyma Ghosh[2], Frank L. Mastaglia[2], Tess Evans[1] and Ryan Anderton

[1]School of Health Sciences and Institute for Health Research, University of Notre Dame Australia, [2]Perron Institute for Neurological and Translational Science

**Introduction.** In the past decade, increased recognition of impulse control disorders (ICDs) in medicated patients with Parkinson's Disease (PD) has raised concordant interest in prodromal trait impulsivity. Several non-motor features of PD are known to adversely affect patient quality of life, however the specific impact of impaired reward and motivation circuitry is yet to be elucidated. **Objective.** The present study investigates the implications of heightened trait impulsivity to quality of life in people with PD. **Procedures.** A total of 328 patients with idiopathic PD were sequentially recruited from three Movement Disorder clinics across Australia. The 39 item Parkinson's Disease Questionnaire (PDQ-39) was used to measure disease specific quality of life, whilst patient impulsivity was determined by Barratt's Impulsiveness Scale Version 11 (BIS-11). **Results.** Patient impulsivity and quality of life scores were found to be significantly associated with one another. When grouped in tertiles of increasing impulsivity, quality of life was consistently subsided in successive groups, with respect to both BIS-11 total and sub domain scores. PDQ-39 scores increased by 48.5% ($t = 17.46$, $p < 0.001$) between low and high BIS tertiles, indicating significantly poorer quality of life. A generalized linear model revealed attentional impulsivity ($\beta = 1.200$, $p = 0.002$) and non planning impulsivity ($\beta = 0.664$, $p = 0.011$) to be predictive of poor quality of life, more so than clinical features such as patient age ($\beta = 0.237$, $p = 0.082$). **Conclusions.** Our findings assert an underappreciated role of impulsivity in the clinical framework of PD, where management of these behavioural disturbances is essential to holistic patient care. Understanding and recognition of critical subclinical symptoms can guide early targeted interventions. Consequent treatment, prior to pathological ICD onset, may ultimately reduce the burden of such symptoms on patient quality of life.

F8

# Rational Design of Short Chemically-modified Antisense Oligonucleotides for Efficient Exon-skipping *in vitro*

Le BT,[1,2] Adams AM,[1] Fletcher S,[1,2] Wilton SD,[1,2] and Veedu RN[1,2]

[1]Centre for Comparative Genomics, Murdoch University [2]Perron Institute for Neurological and Translational Science

**Introduction**. Antisense oligonucleotides (AOs) are prominent class of nucleic acid therapeutic molecules that attracted considerable attention in the last two decades. Recently, Exondys51- a phosphorodiamidate morpholino oligomer (PMO) drug approved by the US FDA for Duchenne muscular dystrophy- has been shown to produce an increase in dystrophin protein and showed an excellent safety profile in the trial participants. In contrast, Drisapersen- a 2'-*O*-methyl (2'-OMe) AO synthesised on a phosphorothioate (PS) backbone- was rejected due to its threatening side effects and poor efficacy. **Problem Statement**. Although PMO monomers are proved to be excellent for clinical usage, it is not compatible with the standard phosphoramidite chemistry, and the current PMO synthesis chemistries are costly. One approach to reduce the costs and possibly limit the toxicity of the 2'-OMePS AOs would be to truncate the AO length, while not compromising the AO efficacy. Towards this, we envisaged the use of locked nucleic acid (LNA), a prominent nucleic acid analogue with high target binding affinity and remarkable stability against nuclease degradation. Introduction of LNA monomers into the truncated 2'-OMePS AO may be a potential strategy in resolving these problems. **Procedures**. In this study, we synthesised and evaluated the efficiency of a series of systematically truncated LNA-modified 2'-OMe AOs on a PS backbone that were designed to induce exon-23 skipping in dystrophin gene transcript in *H-2K$^b$*-tsA58 *mdx* mouse myotubes *in vitro*. **Results**. The results clearly demonstrated that shorter AOs (16 - 14mer) containing LNA nucleotides efficiently induced dystrophin exon-23 skipping compared to the corresponding 2'-OMe AOs. **Conclusions**. Our remarkable findings contribute significantly to the existing knowledge about the design of short LNA-modified AOs for exon-skipping applications. Utilising this approach can help reducing the cost of AOs and potential toxicities of the 2'-OMe PS based oligos.

F9

## Mapping Functional Domains Encoded by the Distal Third of Mouse Dystrophin Gene

Dunhui Li[1,2], Abbie Adams[1,2], Kane Greer[1,2], Kristin West[1,2], Steve Wilton[1,2], Sue Fletcher[1,2]

[1]Centre for Comparative Genomics, Murdoch University; [2] Perron Institute for Neurological and Translational Science

**Introduction**: Duchenne muscular dystrophy (DMD), the most common devastating muscle wasting disorder of children, is caused by *DMD* mutations that cause premature truncation of protein translation. Exondys51®, the first exon skipping drug approved for DMD was developed to change disease progression from the severe DMD form to a Becker muscular dystrophy-like phenotype (BMD), a milder allelic disorder caused by in-frame *DMD* deletions. **Problem Statement**: The distal third of *DMD* encodes several important functional domains, including hinge 4 and the β-dystroglycan and α/β syntrophin binding domains. Single or multi-exon deletions that do not disrupt the reading frame are very rare downstream of exon 55, and hence there is no 'BMD patient guide' to indicate functionality of in-frame exon deletions. **Aim**: We are using splice switching antisense oligonucleotides (AOs) to excise in-frame exon or exon blocks to generate a variety of dystrophin transcripts that can be assessed for function and activity. In this manner we aim to define genotype-phenotype correlations at the resolution of single or multiple in-frame exon blocks in the distal third of *DMD* gene. Any isoforms that retain some degree of function will indicate which mutations may be amenable to therapeutic exon skipping. **Results**: We designed and synthesized 2'-O-Me PS AOs to screen for the most effective compounds to excise exons 56/57 and 58/59 as in-frame exon blocks *in vitro*. Optimal sequences were then synthesized as clinically relevant cell penetrating peptide-conjugated morpholino oligomers. Complete (100%) skipping of both exon blocks was induced in the *mdx* mouse myogenic cells with these more effective compounds. **Conclusions and perspectives**: The exon skipping AOs developed in this study have high efficiency in inducing different *DMD* isoforms and their functions are now being evaluated *in vivo* in mouse models.

F10

## 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]Centre for Comparative Genomics, Murdoch University [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 contained in *ATXN3* and still maintain normal function of the protein. Additionally, significant down-regulation of both the mutant and wild-type protein was observed, allowing for a combination of benefits. Confirmatory data using the clinically relevant phosphorodiamidate morpholino oligomer (PMO) chemistry showed complementary positive results to 2'O-methyl data. However, PMO is widely 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**. Therefore, this study provides a possible therapeutic strategy to treat SCA3.

F11

# Investigating Antisense Oligonucleotide Therapeutics for ALS

Rita Mejzini[1, 2], Prof. Anthony Akkari[1, 2], Prof. Steve Wilton[1, 2]

[1]Centre for Comparative Genomics, Murdoch University, [2]The Perron Institute for Neurological and Translational Science

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease characterised by degenerative changes in 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* and *C9ORF72*) are known to lead to the pathologic aggregation of the 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 cases of ALS, occurring in more than 90% of patients even those without mutations in *TARDBP*. This study has involved the development of antisense oligonucleotides (AOs) designed 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'Omethyl modified bases on a phosphorothioate backbone. Future work will include the evaluation of second generation clinically applicable morpholino oligomers followed by protein analysis, functional assays and evaluation in neuronal models. The development of AOs that selectively target mutated alleles will also be 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.

F12

# Antisense Oligomer Modulation of Selected RNA-binding Proteins to Reduce the Severity of Spinal Muscular Atrophy

Ianthe Pitout[1], Loren Flynn[2], Sue Fletcher[1] and Steve Wilton[1,2]

[1]Centre for Comparative Genomics, Murdoch University, [2]Perron Institute for Neurological and Translational Science

Proximal spinal muscular atrophy is a devastating neurodegenerative disease that is the leading genetic cause of infant death, with a pan-ethnic incidence of 1 in 11,000 live births. Spinal muscular atrophy is most commonly caused by homozygous loss of the survival motor neuron 1 (*SMN1)* gene that encodes an essential protein, SMN. The absence of SMN is embryonic lethal, however humans possess one or more copies of a nearly identical gene, *SMN2* that provides insufficient levels of functional SMN, leading to spinal motor neuron death and severe muscle weakness. A single nucleotide change in *SMN2* exon 7 creates a splice silencer recognition site, leading to the predominant production of truncated mRNA transcripts missing exon 7 that encode a non-functional SMN protein. Pre-mRNA splicing of *SMN2* exon 7 is modulated by numerous RNA-binding proteins, including SAM68 and hnRNP-A1 that promote exon 7 exclusion from the mature transcript. Antisense oligomer-mediated knockdown of SAM68 and hnRNP-A1 therefore, has potential to increase the amount of full-length SMN produced by the sub-optimal *SMN2* gene. Antisense oligomers designed to induce a frame-shift in *SAM68* and *hnRNP-A1,* and thereby mediate their respective protein knockdown, were transfected into spinal muscular atrophy patient cells. Gene transcript and functional protein analysis of transfected cells showed both SAM68 and hnRNP-A1 knockdown that subsequently increased functional SMN protein comparable to levels found to apparently healthy cells. In addition, the lead antisense oligomers targeting *SAM68* and *hnRNP-A1* were evaluated in combination with *Anti ISS-N1*, the current antisense treatment available to spinal muscular atrophy patients. Promisingly, the combination treatments in patient cells showed an additive effect and further increased SMN protein expression. There is thus potential for antisense oligomers targeting SAM68 and hnRNP-A1 to be used as a combinatorial therapy for reducing the severity of spinal muscular atrophy.

# Effects of Daily Low-Intensity Repetitive Transcranial Magnetic Stimulation on Rodent Resting-state Network: A Longitudinal fMRI Study

Bhedita J Seewoo[1, 2, 3], Kirk W Feindel[3, 4] and Jennifer Rodger[1, 2]

[1]Experimental and Regenerative Neurosciences, School of Biological Sciences, UWA; [2]Brain Plasticity Group, Perron Institute for Neurological and Translational Research, Australia; [3]Centre for Microscopy, Characterisation and Analysis, UWA; [4]School of Biomedical Sciences, UWA

Spontaneous fluctuations in brain activity occur in a task-free setting. Brain regions with such coherent spontaneous fluctuations form an organised network called the resting-state network (RSN). Compared to healthy individuals, people with neurological and psychiatric disorders have been identified with RSN dysregulation. Several lines of evidence suggest that repetitive transcranial magnetic stimulation (rTMS), a novel non-invasive neuromodulation technique, is able to modulate the resting-state activity of the brain and that RSN plasticity is sensitive to rTMS in humans. However, the mechanisms underlying its therapeutic effects remains poorly understood. Information about the degree to which the RSN can be modulated by rTMS may prove helpful in the development of treatment options. In this study, we investigated the effects of low-intensity rTMS (LI-rTMS) on the rodent RSN using resting-state functional magnetic resonance imaging (rs-fMRI). Daily 10 min sessions of 10 Hz LI-rTMS were delivered over the right brain hemisphere of nine lightly-restrained Sprague-Dawley rats using a circular stimulation coil over a period of two weeks. rs-fMRI data were acquired at 9.4 Tesla at baseline, after 1 week of stimulation and after 2 weeks of stimulation to determine the cumulative effects of LI-rTMS. We also investigated the duration of these effects by performing two imaging sessions seven days and 20 days after stimulation was ceased. We used independent component analysis and a regression approach to uncover changes in the RSN. LI-rTMS induced a significant increase in functional connectivity following 14 days of stimulation (Day 14 > Day 0). A significant decrease in functional connectivity was observed seven days after stimulation was ceased (Day 21 < Day 14) and following another 13 days of non-stimulation, a further decrease in functional connectivity was noted (Day 34 < Day 14). Our results suggest that daily LI-rTMS stimulation may enable sustained plastic changes in the RSN.

# Artificial Seed Ageing Effects on Short-Lived Orchid Seeds: Comparing Two Orchid Species

Sze-Tieng Ang[1], Bryn Funnekotter[1,2], Ricardo L. Mancera[1], Eric Bunn[1,2]

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

The southwest Western Australia hosts around 400 native orchid species, 99% of which are found nowhere else on Earth. Each orchid seed capsule can produce hundreds to millions of dust-like seeds, so they are the ideal germplasm source for storage. Each orchid seed has an embryo enclosed by a seed coat but does not possess the endosperm that usually provides nutrients for storage. Interestingly, these orchid seeds can be dried to low moisture content and still remain viable without an endosperm. However, they do not retain this viability for very long under normal orthodox storage conditions and are consequently known as short-lived orthodox seeds. For effective seed bank management, seed longevity of each species has to be known and seeds that exhibit short-lived nature will need to be prioritised for more specialised storage techniques, such as cryopreservation. In this study, artificial seed ageing of two orchid species, *Microtis media* (invasive orchid species) and *Caladenia latifolia* (non-invasive orchid species) were undertaken and effects of seed ageing on seeds germination recorded. The seed ageing approach uses high temperature and relative humidity (RH) to accelerate ageing process in the seed. At 35°C and 60% RH, *M. media* had not demonstrated any ageing effect whereas *C. latifolia* lost 20% of its viability after 5-days of ageing process, indicating rapid ageing. When a higher temperature (40°C) and 60% RH were used, *M. media* lost its 20% viability after 20 ageing days while *C. latifolia* showed the same rapid viability lost as in the 35°C ageing experiment. These findings suggest that *M. media* seed has higher temperature tolerance than seed of *C. latifolia*, and provide a useful guide for prioritising future seed conservation research with Western Australian native orchids.



# Characterisation of the Symbiosis ICE and Accessory Plasmid of *Mesorhizobium ciceri* CC1192

Emma Bonello[1], Graham O'Hara[1], Joshua Ramsay[2], Jason Terpolilli[1]

[1]Centre for Rhizobium Studies, Murdoch University, Perth; [2]Curtin Health Innovation Research Institute, Curtin University, Perth.

Symbiosis genes in *Mesorhizobium* spp. are chromosomally-encoded on symbiosis integrative and conjugative elements (ICEs) which are mobile genetic elements capable of conjugative transfer to recipient cells. When *Mesorhizobium* spp. are supplied as inoculants with legumes in the field, this transfer results in the evolution of novel microsymbionts, many of which are sub-optimally effective at fixing $N_2$, indicating that a strong interaction between chromosomal and ICE-encoded genes exists. However, understanding this interaction is currently limited as only one ICE-devoid *Mesorhizobium* recipient exists; *M. loti* R7ANS, produced from the *Lotus*-nodulating parent strain *M. loti* R7A. Furthermore, *Mesorhizobium* spp. may also harbour an accessory plasmid, which may have a role in nitrogen fixation. Recently, the genome of *Mesorhizobium ciceri* CC1192, the commercial inoculant for *Cicer arietinum*, was reported. The CC1192 genome consists of a 6.29-Mb chromosome harbouring a 419-kb symbiosis ICE (ICE*Mc*Sym$^{1192}$) and an accessory plasmid of 648-kb (pMc1192). The aims of this project were to determine the symbiotic role of pMc1192 and to investigate curing the CC1192 genome of ICE*Mc*Sym$^{1192}$. Although pMc1192 harbours a suite of likely symbiosis genes, plasmid-cured derivatives of CC1192 were shown to be unaffected in $N_2$ fixation with *C. arietinum*. To cure CC1192 of ICE*Mc*Sym$^{1192}$, overexpression of the CC1192 *rdfS* was attempted, as a homologous protein previously stimulated ICE excision and loss in *M. loti* R7A. Although *rdfS* overexpression up-regulated excision of ICE*Mc*Sym$^{1192}$, the element was not lost from CC1192. An alternate approach to cure CC1192 of ICE*Mc*Sym$^{1192}$ via homologous recombination was therefore selected and is currently underway. Further work on ICE*Mc*Sym$^{1192}$ will enable the creation of a naïve *M. ciceri* CC1192 strain devoid of the symbiosis ICE and accessory plasmid, providing a valuable research tool to better understand how the symbiotic performance of novel strains is affected by the interaction between chromosomal and ICE-encoded genes.

G3

## Desiccation Issues Surrounding the Cryopreservation of *Syzygium australe*

Hamish Clark[1], Robyn Streczynski[1], Bryn Funnekotter[1,2], Eric Bunn[2], Ricardo Mancera[1]

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

**Introduction**. Recalcitrant species are characterised by high water contents (WC), large seed size, show temperature sensitivity and are metabolically active, making them unsuitable for seed banking. This limits conservation options to storage by cryopreservation of material such as excised embryonic axes. Desiccation to a desirable WC (15-20%) is important for successful cryopreservation, as reduced WC increases the chance of reaching a vitrified or glass state at liquid nitrogen temperatures (-196°C). Historically desiccation has been achieved through air drying or exposure to solutions containing cryo-protective agents (CPAs). Both these treatments can however reduce viability of material. **Problem Statement**. The aim was to investigate reductions in WC against viability of excised axes of *Syzygium australe* in preparation for cryopreservation. **Procedures**. Variables measured were the loss of water from axes through treatments and the resulting viability of the axes. Treatments included sucrose desiccation media, exposure to Plant vitrification solution 2 and 3, and exposure to a dry constant air flow from a laminar flow cabinet to induce desiccation. **Results**. Under flow drying treatments viability was seen to drop rapidly when WC of 25% or less was reached, with viability reducing to as low as 10% once desirable WC were achieved. Neither treatments with PVS2, PVS3 nor desiccation media achieved WC low enough to be considered desirable for cryopreservation. PVS3 reached a WC of 25% but this was with a 40% loss in viability. **Conclusions**. This research acts to establish a base understanding of water loss in embryonic axes of *S. australe*. Results indicate that whole axes cannot be desiccated to water contents suitable for cryopreservation without loss of viability, by means of laminar airflow or CPA desiccation. This understanding is likely applicable to seed axes of other *Syzygium* species as well as recalcitrant rainforest species.

G4

## Free-living and Symbiotic Characterisation of *Burkhlderia sprentiae*

[1]Talitha Rogers, [1]John Howieson, [1]Graham O'Hara, [1]Jason Terpolilli

[1]Murdoch University, Perth, Western Australia

*Lebeckia ambigua* is a papilionoid legume endemic to the Western Cape region of South Africa. As it is well-adapted to dry, sandy and acidic soils, *L. ambigua* is being evaluated as a perennial forage legume for mixed farming systems in parts of the south west of Australia. *L. ambigua* forms symbiotic assoications with *Burkholderia* spp., however Australian soils appear to lack rhizobia capable of fixing nitrogen with this legume. Therefore, a range of *Burkholderia* strains isolated from South Africa are also being evaluated as potential commercial inoculants for this legume. At present, very little is known about the genetics and physiology of the papilionoid *Burkholderia,* which limits the development of these strains as robust and efficient commercial inoculants. We have recently completely sequenced the genome of *Burkholderia sprentiae* WSM5005 which consists of a chromosome (3.6 Mbp), a large plasmid (pBs1, 2.66 Mbp), plus three smaller plasmids (pBs2, pBs3 and pBs4, at 996 Kbp, 439 Kbp, and 9.5 Kbp, respectively). Most nodulation (*nod*) and nitrogen fixation (*nif* and *fix*) genes are encoded on the largest plasmid pBs1, while pBs2 consists of a range of genes, of which half are uncharacterised, hypothetical proteins. There are duplicates of several *nod* genes located on pBs3, along with a high proportion of uncharacterised genes. Genes located on pBs4 are predominantly hypothetical proteins. Of note is the absence of genes encoding the $cbb_3$-type cytochrome oxidase (*fixNOQP)*, known to play an integral role in microaerobic growth and symbiotic nitrogen fixation in characterised α-rhizobia. In its place, WSM5005 appears to carry two complete cytochrome *bd*-type terminal oxidases (*appBC, cydAB*), which presumably functions to support microaerobic and symbiotic metabolism. Further work is characterising the symbiosis between *L. ambigua* and WSM5005 and transcriptionally-mapping the strain in free-living and nitrogen-fixing conditions.

# Investigating the Stability and Evolution of *Mesorhizobium* Symbiosis Integrative and Conjugative Elements

Georgina Stagg[1], Graham O'Hara[1], Josh Ramsay[2], Jason Terpolilli[1]

[1]Centre for Rhizobium Studies, Murdoch University, [2]School of Biomedical Sciences and Curtin Health Innovation Research Institute, Curtin University

**Introduction**. Rhizobia are soil dwelling bacteria that form symbiotic associations with legumes, whereby they reduce inaccessible atmospheric nitrogen ($N_2$) into the bioavailable ammonia. Rhizobia within the *Mesorhizobium* genus harbour genes essential to the establishment and maintenance of $N_2$ fixing-legume symbioses on chromosomally-encoded mobile segments of DNA known as symbiosis integrative and conjugative elements (ICEs). Symbiosis ICEs can transfer to recipient strains devoid of these elements, integrating into the host chromosome at conserved attachment sites, yielding novel strains capable of forming symbiotic interactions. Analysis of symbiosis ICEs has revealed that many share core regions of homology, suggesting that recombination between symbiosis ICEs may be common. **Problem Statement**. Recombination between symbiosis ICEs is most likely to occur between two or more elements harboured within the same strain. However, transfer of these elements between ICE-harbouring strains has not been tested. **Procedures**. Two strains, *M. loti* R7A and *M. ciceri* CC1192, harbouring two genetically distinct elements with different attachment sites were selected to be marked with *W-aadA* and *nptII,* respectively. The marked ICEs will be mobilised via a conjugation mating into a recipient strain harbouring its own unique ICE in reciprocal experiments. The outcome of this experiment will determine if two genetically distinct symbiosis ICEs can coexist, or whether the presence of one ICE precludes the adoption of a second element in a recipient strain. **Results**. Both strains, *M. loti* R7A and *M. ciceri* CC1192 have been marked with their appropriate marker and the symbiotic effectiveness of these marked strains are being assessed in a glasshouse study. The conjugation experiment between the two marked strains are currently underway. **Conclusions.** ICEs are a fundamental part of the bacterial mobilome, understanding how they interact will impart crucial information on their stability and evolution.



# The Use of Antioxidants to Increase Survival of *Syzygium* Meristems During the Cryopreservation Process

Robyn Streczynski[1], Bryn Funnekotter[1,2], Ricardo Mancera[1]

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

**Introduction.** Recalcitrant rainforest species such as *Syzygium* cannot be stored under conventional seed banking conditions for conservation. Hence, there is a need to develop cryopreservation protocols that allow for their survival following storage in liquid nitrogen (-196°C). This process involves desiccation to reduce water content through either air drying or using osmotic medium, followed by exposure to cryo-protective agents (CPAs) to promote vitrification, whereby the plant material can then be cryopreserved. **Problem statement.** Oxidation however, is a major issue during the cryopreservation process and impacts on the survival of the plant material used. Oxidative stress occurs due to an imbalance between prooxidants and antioxidants, either from decreased antioxidants or increased reactive oxygen species (ROS). This can occur during any of the steps throughout the cryopreservation process. The aim is therefore to test the addition of antioxidants to mitigate oxidative stress. **Procedures.** Antioxidants including glutathione (GSH) and ascorbic acid (AsA) were added to 0.4 M sucrose desiccation medium (preculture step) in different amounts to determine the optimal concentration where survival is the highest. The meristems of *Syzygium australe* were used for these experiments. FDA staining was also used in fluorescence microscopy to show increased survival with added antioxidants compared to controls. **Results.** Meristems on 0.4 M sucrose media with 5 mM GSH incubated for 3 days, showed 100% survival, and meristems on 0.4 M sucrose media with 50 mM AsA incubated for 3 days, showed 71% survival. Compared to a control of 0.4 M sucrose media without antioxidants incubated for 3 days, showed only 57% survival. **Conclusions.** Increasing survival during the steps of cryopreservation is necessary for the development of successful protocols. The addition of antioxidants to the medium reduced oxidative stress and improved survival rates of *S. australe* meristems at key stages in the cryopreservation process.

## Genetic Diversity and Distribution of Filamentous Prophage in *Neisseria*

Barakat Alsuwayyid[1,2], Geoffrey Coombs[3,4], David Speers [3,5], Julie Pearson[3], Michael Wise[1,6], Leah Rankine-Wilson[1] and Charlene Kahler[1]

[1]The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Sciences, UWA, [2]Ministry of Education, Saudi Arabia. [3]Pathwest Laboratory Medicine [4]School of Veterinary and Life Sciences, Murdoch University. [5]School of Medicine and Pharmacology & [6]Computer Science and Software Engineering, UWA.

A filamentous bacteriophage termed the Meningococcal Disease Associated (MDA) phage is associated with *Neisseria meningitidis* clades which cause invasive meningococcal disease. MDAφ improves mucosal colonization of the nasopharynx by meningococci and thus increasing the incidence of bloodstream invasion associated with meningococcal carriage. We recently recovered a gonococcal isolate (ExNg63) from a rare case of gonococcal meningitis and whole genome sequencing revealed that this isolate possessed a region with 90% similarity to MDAφ found in *N. meningitidis*. This is the first indication that MDA-like prophage may not be restricted to *N. meningitidis*. Therefore, to understand the genetic diversity and distribution of MDA-like prophage, we examined the distribution, prevalence and genetic diversity of filamentous phage in Neisseria. Closed genomes of 44 *N. meningitidis*, 28 *N. gonorrhoeae*, 2 *N. lactamica* and 17 commensal *Neisseria* species were collected from the NCBI database. Filamentous prophages were defined as a set of genes that have the size and genetic organization similar to the MDAφ in *Neisseria meningitidis* Z2491 or Ngo6-8 in *Neisseria gonorrhoeae* FA1090. A maximum likelihood phylogenetic tree was constructed using MEGA7 while heirBAPS was used to define genetic population groups of prophages. One hundred and sixty filamentous prophages were detected in the dataset and population structure analysis revealed that the putative gonococcal MDA-like prophage and a putative MDA-like prophage in *N. lactamica* formed a structure group with meningococcal MDAφ. However, only 7.5% of gonococcal isolates available at BIGSdb possessed a complete or partial MDA-like sequence compared to 46% of meningococcal isolates suggesting that acquisition of MDA-like prophage is rare in this species. These data suggest that prophages similar to the meningococcal MDAφ are present in *N. gonorrhoeae* and *N. lactamica* and more work is required to determine whether MDA-like phage act as accessory colonization factors in these species.



## 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

**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.

# Does PITP dependant PtdIns-4-P production promote encystation in *Giardia duodenalis*

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

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

**Introduction.** *Giardia duodenalis,* an intestinal parasite has a simple two step life-cycle; the vegetative trophozoite and the environmentally resistant cyst. The cyst is produced through the process of encystation. This process involved the production, synthesis and secretion of cyst wall proteins (CWPs) forming the extracellular matrix conferring environmental resistance to the cyst. The trafficking of these CWPs is via encystation specific vesicles (ESVs) and is the only known regulated export pathway in *G. duodenalis.* In an attempt to identify factors that coordinate encystation whole cell proteomic analysis identified expression of a putative phosphatidylinositol transfer protein (PITP) to be elevated during encystation. Nothing is known about PITP function in *G. duodenalis,* however, studies in other organisms suggest that this expression profile is not coincidental. **Problem Statement.** We hypothesise *Gd*PITP dependent PtdIns-4-P production to be essential for the trafficking of CWPs to the cell periphery during encystation. **Procedures.** *G. duodenalis* assemblage A was induced to undergo encystation for 24 hours. RNA was extracted every 3 hours from the cells and *Gd*PITP expression determined by qPCR. Secondly, GdPITP (A) was cloned and heterologous expression vectors constructed to allow for characterisation of PITP activity. **Results.** We have verified PITP gene expression to be induced during encystation. Heterologous expression of GdPITP (A) in yeast shows it to be toxic to several mutants defective in PtdIns-4-P synthesis in a manner reminiscent of that shown for the sterol regulated PITP Sfh3. **Conclusions.** Little is known about the molecular mechanisms that control encystation in *G. duodenalis*. Understanding how *Gd*PITP is involved in the mechanisms of encystation, may act as a potential target for pharmaceutical intervention, diminishing the ability of *G. duodenalis* to undergo encystation, therefore ablating environmental resistance.



# Should Australia Still be Considered Free from *Hepatozoon canis*?

Telleasha L Greay[1], Amanda D Barbosa[1], Robert L Rees[2], Andrea Paparini[1], Una M Ryan[1], Charlotte L Oskam[1] and Peter J Irwin[1]

[1]Vector and Waterborne Pathogens Research Group, School of Veterinary and Life Sciences, Murdoch University, [2]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne

**Introduction**. Recent molecular and serosurveillance studies of the tick-borne pathogen *Hepatozoon canis* have identified new hosts, potential vector species, and have revealed that *H. canis* is more widespread than previously thought. During a survey for apicomplexan pathogens in ticks from companion animals in Australia, *H. canis* was unexpectedly detected in a single Australian paralysis tick (*Ixodes holocyclus*). As there are no previous reports of this pathogen in Australia, it is currently considered exotic to Australia. **Problem Statement**. We hypothesised that the presence of *H. canis* in the engorged *I. holocyclus* tick was a result of infected blood meal from the canine host, and that the host was infected with *H. canis*. **Procedures**. A blood sample was collected from the host of this tick, a Maremma Sheepdog in Sarina, Queensland. Blood smears were prepared and morphologically assessed for the presence of *H. canis*, and conventional PCR was used to amplify *H. canis* 18S rDNA (18S) in genomic DNA that was extracted from the blood sample. Sanger sequencing was used to determine the sequence identity of the conventional PCR product. **Results**. Blood smear examination and a 1,413 bp 18S sequence (GenBank® accession no. MG062866) confirmed that the dog was infected with *H. canis*. The 18S sequence was also 100% identical to *H. canis* isolated from *I. holocyclus* (MG758124). **Conclusions**. This finding represents the first diagnosed case of canine hepatozoonosis in Australia. Biosecurity authorities were notified, and the results were confirmed by the Australian Animal Health Laboratory in Geelong, Victoria. It is unknown when or how the organism was introduced into Australia, which raises questions about border biosecurity policies and the *H. canis* infection status of its potential vectors and hosts in Australia. Further surveillance for this pathogen is required to determine whether *H. canis* has established in Australia.

# Persistence of Dengue and Chikungunya Virus Co-circulation Following a 2015 Dengue Outbreak in Jambi, Indonesia

H. Harapan[1,2], S. Haryanto[3], R.T. Sasmono[4], D. Smith[1,5], A. Imrie[1,5]

[1]School of Biomedical Sciences, University of Western Australia, [2]Medical Research Unit, Syiah Kuala University, Indonesia, [3]Siloam Hospital, Indonesia, [4]Eijkman Institute, Indonesia, [5]Pathwest Laboratory Medicine, Australia

In 2015, a dengue outbreak occurred in Jambi municipality in Sumatra island of Indonesia and co-circulation DENV, chikungunya (CHIKV) and Zika virus (ZIKV) was identified. To monitor ongoing transmission, a molecular epidemiology study was conducted in Jambi in 2016. One hundred and forty two sera from patients with dengue-like illness (DLI) were tested for DENV antigen by NS1 ELISA and RT-PCR and inoculated onto Vero cells to isolate virus. Sera excluded for DENV were assessed for leptospirosis IgM and IgG and for ZIKV genome by RT-PCR. During a one year period, 21 DENV infections were identified caused by DENV-1, -2 and -3; DENV-2 was the predominant serotype. Possible leptospirosis infection was identified in 2 out of 116 sera and CHIKV infection was detected by RT-PCR in 3 sera. Maximum likelihood phylogenetic analysis of DENV E and CHIKV E1 gene sequences showed that all DENV-1 grouped as Genotype I; DENV-2 as Cosmopolitan genotype; and DENV-3 as Genotype I, indicating no DENV genotype shift for all three serotypes for two consecutive years (2015-2016). All CHIKV isolates belonged to Asian genotype, the same genotype that circulated the previous year. Altogether, we show that DENV and CHIKV are endemic in Jambi and co-circulated during a 2-year period in 2015-2016. Therefore, specific testing is required to exclude CHIKV infection in patients with DLI in the clinical setting and systematic surveillance should be implemented to decrease misdiagnosis and under-reporting of chikungunya in this region.



# Investigation of the Causative Agent Associated with Two National-wide Chikungunya Outbreaks in Indonesia, 2009-2010 and 2013

H. Harapan[1,2], S. McCarthy[1], A. Michie[1], T. Ernst[1], M. Mudatsir[2], R. Nusa[3], B. Yohan[4], R.T. Sasmono[4], D. Smith[1,5], A. Imrie[1,5]

[1]School of Biomedical Sciences, University of Western Australia, Australia, [2]Medical Research Unit, Syiah Kuala University, Indonesia, [3]Ministry of Health of the Republic of Indonesia, Indonesia, [4]Eijkman Institute, Indonesia, [5]Pathwest Laboratory Medicine, Australia

Chikungunya re-emerged in Indonesia in early 2001 and since then multiple and sporadic outbreaks have occurred in the country. Two nation-wide outbreaks have been reported, during 2009-2010 and in 2013 with 137,655 and 15,324 cases, respectively. The Chikungunya virus (CHIKV) genotypes associated with these distinct outbreaks have not been well identified. We conducted a systematic review, temporal-spatial analysis, and phylogenetic and evolutionary analysis of Indonesian CHIKV to identify circulating genotypes. In addition, archival sera from Western Australia (WA) travelers returning from Indonesia during the peak of 2013 outbreak were tested for CHIKV IgM and/or RT-PCR, and CHIKV E1 gene was sequenced. Whole genomes of representative CHIKV were sequenced using the Illumina platform. Bayesian evolutionary analysis was conducted using MCMC method as implemented in BEAST. Our systematic review identified 129 Indonesian CHIKV sequences (from 1983 to 2015), of which 92.3% were Asian genotype and 7.7% belonged to the ECSA genotype. ECSA viruses were likely introduced to Indonesia from a neighboring country in 2008, were continually sampled until 2011 and disappeared afterward. All ECSA viruses sampled in Indonesia were closely related to viruses that caused major outbreaks in Southeast Asia countries during the same period. These phylogenetic and evolutionary data together with our spatial analysis suggest that the 2009-2010 outbreak was due to introduction of the ECSA genotype to Indonesia. Analysis of archival 2013 WA traveler sera showed 11 CHIKV grouped as Asian genotype and were closely related to endemic CHIKV that have circulated in Indonesia since 2004. In conclusion, two nation-wide chikungunya outbreaks occurred in Indonesia in close proximity, in 2009-2010 likely caused by CHIKV ECSA genotype and in 2013 likely caused by CHIKV Asian genotype. These data indicate rapid genotype shift during 2011-2012. Continued chikungunya surveillance including molecular epidemiological analysis should be implemented in Indonesia.

# Accelerometers Forecast Behavioural Response to Climate Change in Critically Endangered Sawfish

Karissa Lear, David Morgan, Jeff Whitty, Adrian Gleiss

Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University

The Fitzroy River in the Kimberley region of Western Australia represents one of the last intact nursery habitats for the critically endangered freshwater sawfish (*Pristis pristis*). However, this region is under increasing threats from both climate change and land use actions, which include proposals for significant water extraction to fuel agricultural development. Both of these factors have the potential to substantially change the physical characteristics of this crucial nursery habitat, including river flow rates and water temperatures. Understanding how sawfish will respond to these changing environmental variables is thus crucial to informing conservation measures for these animals and their unique ecosystem. However, this can be a difficult task, as the effectiveness with which animals respond to environmental change depends on a complex interaction between how temperature and other factors change their physiology and behaviour, as well as the degree of behavioural plasticity involved in this response. Here we use a combination of respirometry and accelerometry methods to decipher patterns of behaviour and energy use in free-ranging sawfish. Field energy expenditure, activity levels, and body condition are compared between multiple years within 2011-2017 characterized by significant wet seasons and resulting high water levels, flow rates, and generally lower temperatures, and by poor wet seasons, low flow rates, and higher water temperatures. Results are discussed in the context of forecasting how sawfish may respond to climate change and land development in the Fitzroy region, and informing management and conservation measures for this critically endangered species.



# An Investigation into the Blood-Borne Parasites Infecting Tasmanian Devils (*Sarcophilus harrisii*)

Sarah Munns [1], Amanda Barbosa[1], Manuel Ruiz-Aravena[2], Menna Jones[2], Charlotte Oskam[1], Una Ryan[1]

[1]Vector and Waterborne Pathogens Research Group, School of Veterinary and Life Sciences, Murdoch University, [2]University of Tasmania, Hobart, Tasmania

**Introduction**. The Tasmanian devil (*Sarcophilus harrisii*) is an iconic carnivorous Australian marsupial on the brink of extinction due to the infectious devil facial tumour disease (DFTD). Tasmanian devils are also host to two native tick species (*Ixodes fecialis* and *Ixodes tasmani*) and previous studies have indicated that these ticks have the potential to transmit disease causing microorganisms, including blood-borne parasites. **Problem Statement**. The aims of this study are to identify and characterise tick associated blood-borne parasites (Apicomplexa) from wild Tasmanian devils and their ticks. **Procedures** Blood and tick samples from 140 Tasmanian devils from 2 sites on the Tasmanian main land (Takone and Wilmot) were collected between 2015 and 2018. Genomic DNA was extracted from a subset of 137 ticks and screened by PCR using Apicomplexan-specific primers targeting ≈ 1500 bp region of the 18S rRNA gene. PCR positive samples were Sanger sequenced for species identification and phylogenetic analysis, and were further validated by microscopic examination of the corresponding blood smears. **Results**. Here we identified the presence of *Hepatozoon* sp. (GenBank ® accession no. of closest match MG758137.1, and 100% identity) and *Theileria* sp. (GenBank ® accession no. of closest match, MG 758115.1 and 100% identity) in 40% (55/137) and 5% (4/76) of the ticks, respectively. **Conclusions**. This study has confirmed the potential for native ticks to act as transmission vectors of blood-borne parasites to Tasmanian devils. The presence of Apicomplexan parasites in ticks and Tasmanian devils is intriguing and may influence Tasmanian devils susceptibility to DFTD. This research provides a new direction for aiding the conservation efforts of the Tasmanian devil.

# A Small Problem in Alpacas

Craig Sergeant[1], Matthew Jones[1], Carolyn Oddie[1], David Groth[1], and Kylie Munyard[1]

[1] CHIRI, Curtin Institute for Computation, School of Pharmacy and Biomedical Science, Curtin University

An alpaca (*Vicuna pacos*) breeder in New Zealand noticed that one his alpacas was smaller than expected and sired smaller than expected offspring approximately 50% of the time. The size of an alpaca directly affects productivity and profit for farmers because alpacas are farmed for fibre and meat. If the cause of these smaller alpacas was able to be identified, the affected animals can be either removed from herds and breeding programs to prevent negative impacts on profit, or they could be bred for niche commercial (i.e. smaller landholdings) or domestic uses (i.e. as pets). Body measurements and weights were collected from the original small alpaca, his offspring, the offsprings' dams, and unrelated alpacas (n= 14) along with pedigree information and blood samples for genetic testing. The affected animals had a shortened spine (p= 0.006), metacarpus (p= 0.02), femur (p= 0.007) and metatarsus (p= 0.02). As the radius and tibia did not differ in length significantly this corresponds to a disproportionate dwarfism phenotype. Dwarfs were also significantly lower in weight than normal animals (5.3 - 8.5 kg dwarf compared with 7.5 - 10.6 kg normal, n= 11, p= 0.01). Using pedigree information, the dwarfism was identified to be autosomal dominant (p= 0.0024) with no evidence to support that this is a sex-linked trait (p= 0.65). Genomic DNA (n=14, 5 affected 9 unaffected) is currently being processed via whole genome novaseq sequencing at Deakin University (Genomics Research and Discovery Facility). A GWAS to identify candidate causative QTL will be conducted.

# Diversity Analysis of Reef Building Coral through Environmental DNA

Jason B. Alexander[1], Zoe Richards[1], Michael Bunce[1]

[1]Department of Environment and Agriculture, Curtin University of Technology

Coral reef systems, home to approximately one third of described marine life, are highly susceptible to environmental changes. With severity and frequency of disturbances such as thermal stress events increasing, the ability to accurately and efficiently census coral biodiversity is integral in management and conservation planning. This study aims to determine the efficacy of environmental DNA, or eDNA metabarcoding technologies, as a new method of detecting coral biodiversity. Environmental DNA samples were collected at 10 sites at the Cocos (Keeling) Islands, with nine replicated surface water and sediment samples collected along three transects at each site. The resultant data was compared to visual biodiversity surveys completed along the same transects. Selected primers targeted the highly variable ITS2 region, which is a commonly used barcoding gene with reference datasets available in GenBank. Paired-end sequencing was undertaken on both the variable ITS2 barcode regions, using an Illumina Miseq system. Results are pending; however a preliminary analysis of water samples demonstrates that the ITS2 region can be successfully amplified from surface water samples. We detected 23 genera of scleractinan corals from the Cocos (Keeling) Islands, compared to the 29 genera that were recorded on visual surveys. Subsequent in-silico investigation has revealed that the existing primer set is unable to bind to species of the genus *Acropora* and *Platygyra*. We infer multiple primer sets are required to elucidate generic and species diversity across the entire scleractinian web of life. As a result, additional primers targeting both the ITS2 and 16S regions are currently being trialed.



# Marine Environmental DNA Reveals Spatial and Temporal Community Changes in Zooplankton

Tina E Berry[1], Megan L Coghlan[1], Benjamin J Saunders[2], Matthew Power[1], Michael Stat[1,3], Simon Jarman[1], Anthony J Richardson[4,5], Claire H Davies[4], Oliver Berry[7], Euan S. Harvey[2] and Michael Bunce[1]

[1]Trace and Environmental DNA (TrEnD) and [2]Fish Ecology, Curtin University; [3]Biological Sciences, Macquarie University; [4]CSIRO Oceans and Atmosphere; [5]Centre for Applications in Natural Resource Mathematics, The University of Queensland; [6]CSIRO Environomics.

**Introduction**. Zooplankton form highly biodiverse communities that are the trophic links between phytoplankton and larger predators. The composition of zooplankton populations are known to fluctuate in response to biotic and abiotic triggers. Consequently zooplankton are used for oceanic biomonitoring. Historically morphological methods have been used to meet this need, but morphological identification of zooplankton is both time consuming and expensive. **Problem Statement**. There is increasing recognition that morphology alone will struggle to meet the current and future needs of biomonitoring in marine conservation and management decisions. There is a need for an adjunct that is fast, affordable and provides the capacity to detect a range of taxa, particularly taxa that is unseen using morphological methods. **Procedures**. Here we use plankton samples to design and test over twenty novel assays, while additionally comparing previously described assays. The aim; to devise a multi-gene metabarcoding 'tool kit', capable of detecting a wide range of zooplankton taxa from environmental DNA. Assays from the 'tool kit' were applied in two separate studies, using both spatial (Australia wide; across three years) and temporal (Rottnest Island; across five years) samples, to test their ability to map changes in response to climate and location. **Results**. Many hundreds of taxa were identified within both studies. Yet genetic reference database limitations prevented identification of over a third of the sequences queried. The data was subsequently examined by forming operational taxonomic units (OTUs: a taxonomy free method to include all genetic data). The OTU analysis demonstrated clear spatial differences in zooplankton communities around Australia and mapped significant changes in the makeup of the Rottnest community in response to seasonality and heatwave stress. **Conclusions**. These studies provide extensive evidence for the application of multi-gene metabarcoding methods to environmental DNA in long term biomonitoring programs.

# Molecular Barcoding of Australian Ticks

Megan Evans[1], Siobhon Egan[1], Peter Irwin[1], Charlotte Oskam[1]

[1]Vector and Waterborne Pathogens Research Group, School of Veterinary and Life Sciences, Murdoch University

**Introduction**. Recently, our research group has characterised the microbiomes of several Australian ticks and have identified potentially pathogenic microorganisms, which may cause disease in animals and humans. However, tick identification is an essential component to attributing these microorganisms to certain tick species and current morphological keys do not provide the required detail to definitively assign taxonomy to species level. This is due to morphologically similar species, morphological keys that are absent for larvae and nymphal ticks, or when morphological features are damaged. Therefore an alternative methodology is required. Molecular barcoding of Australian tick species would allow for more accurate identification of ticks at all life stages and tick conditions. **Problem Statement**. The aims of this project were to identify the ideal gene(s) for use in molecular barcoding of native Australian tick species and determine the optimal PCR assays to discriminate between species. **Procedures**. Four candidate genes (COI, ITS2, 16S rRNA, and 12S rRNA) were investigated for use in tick barcoding. Genes were amplified using PCR, products were separated using gel electrophoresis and Sanger sequenced. Phylogenetic analyses were employed to assess the ability of the DNA barcodes to discriminate between species. **Results**. Currently samples representing all four genera of native Australian ticks have been screened using each optimised barcoding assay. COI was amplified in 92.6% of samples (n=54), ITS2 in 92.3% (n=14), and 16S and 12S have so far been amplified successfully in 100% of samples (n=11 and n=9 respectively). Screening of samples is ongoing and thorough phylogenetic analyses will follow. **Conclusions**. This study has described barcoding assays that amplify target barcoding genes with high rates of success. Furthermore, it has obtained genetic sequences for genes that have not previously been sequenced in many species, filling in gaps in available tick genetic data. The results of this study provide valuable reference material for research that requires accurate identification of Australian ticks.



# Using Invertebrate DNA (iDNA) Metabarcoding to Track rRestoration Trajectories of Arthropods at Mine Sites.

Kristen Fernandes[1,2], Mieke van der Heyde[1,2], Megan Coughlan[2], Grant Wardell-Johnson[1], Michael Bunce[2], Richard Harris[3], and Paul Nevill[1]

[1]ARC Centre for Mine Site Restoration, Curtin University; [2] Trace and Environmental DNA (TrEnD) Laboratory, Curtin University; [3]School of Molecular and Life Sciences, Curtin University

Monitoring the biotic environment is a crucial component of ecological restoration, facilitating baseline understanding of ecological condition, and determining restoration trajectories. Invertebrate monitoring is one approach that has been used to assess ecosystem function, but is underutilised routinely due to the high levels of expertise, time, and cost required. Invertebrate DNA (iDNA) metabarcoding has been successfully applied in the characterisation of arthropod biodiversity but has yet to be applied in the context of environmental restoration. Here, we evaluate the ability of iDNA metabarcoding of bulk arthropod collections to detect changes in community composition. Focusing on two mine site restoration chronosequences in south-western Australia, invertebrates from pitfall traps at different age plots were profiled using two arthropod COI and a plant trnL assay. This allowed for the characterisation of not only arthropods, but the plant DNA that they carried with them from the environment. The metabarcoding data revealed arthropod community assemblages present at the sites and could detect significant differences between these communities within both the chronosequences. This showed that arthropod community assemblages trended towards reference communities as the restoration got older, complementing the results from vegetation surveys from the same age plots. Several taxa were identified as significant in characterising age groups within the chronosequence including; ants (Family: Formicidae), a key bioindicator taxa in Australia; springtails (Order: Collembola) and millipedes (Order: Julida). This study represents the first step in development of an iDNA molecular 'toolkit' for monitoring of ecological restoration projects. Importantly, our results demonstrate that a high throughput iDNA metabarcoding approach, even at this early stage of development, can already complement existing non-molecular monitoring practices. The further development of this technique will allow for fine resolution monitoring of a range of invertebrates over wider geographic and temporal scales than traditional morphology based studies have been capable of.

# Evaluation of 16S and 18S rRNA Next-generation Sequencing for Parasite and Bacterial Pathogen Identification in Wastewater Samples

Telleasha L Greay[1], Alireza Zahedi[1], Alexander Gofton[1], Andrea Paparini[1], Kathryn L Linge[2,3], Cynthia A Joll[3], Arron Lethorn[4] and Una M Ryan[1]

[1]School of Veterinary and Life Sciences, Murdoch University, [2]Curtin Water Quality Research Centre, Curtin University, [3]ChemCentre, [4]Water Corporation of Western Australia

**Introduction**. Recycled wastewater has the potential to carry microbial pathogens, such as viruses, bacteria, protozoa and helminths, which can be a major risk to public health. **Problem Statement**. This study characterised the bacterial and eukaryotic sequence compositions of four wastewater treatment plants (WWTPs) and evaluated the ability of 16S V4 NGS and 18S V9 NGS to identify parasites and bacterial pathogens. **Procedures**. Three WWTPs that utilise stabilisation ponds and one activated sludge plant were sampled. V4 and V9 hypervariable regions of bacterial 16S and eukaryotic 18S were targeted for next-generation sequencing (NGS) on the Illumina MiSeq platform, respectively. Taxonomy was assigned to the sequences using QIIME 2 and the 16S Greengenes and 18S SILVA databases were used for taxonomic assignment. The eukaryotic and bacterial taxa assignments were cross-checked with the National Center for Biotechnology Information non-redundant nucleotide (NCBI nr/nt) database. **Results**. Twelve bacterial pathogens were assigned taxonomy by the Greengenes database, but comparison of sequences from genera and families known to contain pathogens to the NCBI nr/nt database showed that only three pathogens (*Arcobacter venerupis*, *Laribacter hongkongensis* and *Neisseria canis*) could be confidently identified at the V4 region. Importantly, Enterobacteriaceae genera could not be differentiated. Family level taxa assigned by the Greengenes database agreed with NCBI nr/nt in most cases, however, BLAST analyses revealed erroneous taxa in the Greengenes database. For 18S NGS, *Endolimax* spp., *Entamoeba* spp., *Iodamoeba* spp., the human pinworm (*Enterobius vermicularis*) and six *Blastocystis* sp. subtypes were detected. However, *Cryptosporidium*-specific NGS was required for *Cryptosporidium* detection in all WWTPs. **Conclusions**. This study highlights the importance of validating taxonomy of amplicon NGS sequences with the NCBI nr/nt database and recommends including the V3 region of 16S to identify bacterial enteric pathogens. We have also demonstrated that more specific NGS approaches are required for eukaryotic pathogen detection.



# Molecular Detection and Epidemiological Features of Selected Bacterial, Viral and Parasitic Enteropathogens in Stool Specimens from Children with Acute Diarrhoea in Iraq

Ali Harb [1, 2,3], Sam Abraham [1,2], Bertha Rusdi [1,2], Tanya Laird [1,2], Mark O'Dea [1,2], and Ihab Habib[1,2]

[1] School of Veterinary and Life Sciences &;[2] Antimicrobial Resistance and Infectious Diseases (AMRID) Laboratory, Murdoch University;[3] Thi-Qar Public Health Division, Ministry of Health, Iraq

Knowledge of etiology that causes diarrheal illness is essential to implement public health measures to prevent and control this disease. Published studies regarding the situation of diarrhoea in children aged <5 years in Iraq is scanty. This study aims to investigate, using molecular methods (qPCR, RT-PCR, nested PCR and WGS), the occurrences of bacterial (*Salmonella* spp., and *Campylobacter* spp.), viral (Adenovirus, Norovius GI and GII, and Astrovirus) and parasitic (*Entamoeba* spp., and *Giardia* spp.) etiology, and their related epidemiological features, in stool samples from 155 children diarrhoeal cases enrolled between March and August 2016 in a hospital-based cross-sectional study in Thi-Qar Governorate, south-eastern Iraq. Adenovirus was the most frequently detected enteropathogen [53/155 (34.2 %)], followed by *Salmonella* spp. [23/115 (14.8%)], then *Entamoeba* spp. [21/155 (13.5%)], and *Campylobacter* spp. [17/155 (10.9%)]. Mixed-infection between *Salmonella* spp. and *Campylobacter* spp. was evident, and the same was revealed between various enteric viruses, in particular adenovirus and norovirus. The most frequent co-infection pattern was between adenovirus and *Campylobacter* spp., in seven cases [7/155 (4.5%)]. Whole-genome sequencing (WGS) analysis of two adenovirus PCR positive samples using SPAdes de novo assembly of raw reads returned large contigs consistent with HAdV-41 adenovirus genomes. In addition, WGS derived typing data for *Salmonella* isolates (n= 23) revealed that sequence type 49 was the most presented [15/23 (65.2%)] in the tested samples of children diarrhoeal cases. This study provides the first report on detection and identification of *flo*R, $bla_{\text{CARB-2}}$ and *mph*A antimicrobial resistance genes in *Salmonella* isolated from children in the Middle East region. Logistic regression analysis concluded few enteropathogen-specific correlations between children age, household water source, and breastfeeding patterns in relation to the outcome of detection some individual enteropathogens. This study provides supporting evidence for planning of childhood diarrhoea management programs. It is important to build on this study and plan for future longitudinal case-control research in order to investigate in details the epidemiology of enteropathogens in childhood diarrhoea.

# A SNP in the *KIT* Gene is Responsible for the Classic Grey Phenotype in Alpacas (*Vicugna pacos*)

Matthew Jones[1] Craig Sergeant[1], Carolyn Oddie[1], David Groth[1], Samantha Brooks[2], and Kylie Munyard[1].

[1] CHIRI, Curtin Institute for Computation, School of Pharmacy and Biomedical Sciences, Curtin University[2] Brooks Equine Genetics Lab University of Florida Genetics Institute, University of Florida USA

**Introduction:** The genetic mechanisms of alpaca patterning are not well known. One pattern of particular interest is classic grey. Depending on the breeder, this pattern can be either desirable or non-desirable. . This phenotype has been linked to the blue eyed white (BEW) phenotype, which is associated with deafness and reproduction problems. The development of a test for this phenotype would be beneficial for many reasons: firstly, it would allow breeders to efficiently select for a desired phenotype; secondly, it would limit the occurrence of the BEW animals to only those breeders who select for it. **Problem statement:** We have discovered the mutation responsible for the classic grey pattern in alpacas. Breeders will soon be able to use this information to avoid producing unwanted BEW. **Procedures:** The entire KIT cds (21 exons) was sequenced in 6 alpacas; 3 grey and 3 black. A non-synonymous SNP in exon 3 was heterozygous in all classic grey animals, and homozygous wild-type in the black animals, conforming to the mode of inheritance for the classic grey pattern. A novel adaptation of tetra-primer ARMS PCR was used to genotype this SNP in >250 animals. **Results:** A SNP in one of the KIT exons is in perfect association with the classic grey phenotype in alpacas (Patent Pending). Of the >250 animals genotyped, all phenotypically classic grey animals had the mutation. BEW animals also genotyped as classic grey, which confirms that the classic grey phenotype plays a role in the BEW phenotype. There were no animals that were homozygous for the mutant allele, confirming that the pattern is homozygous lethal. **Conclusions:** We have shown that the classic grey phenotype in alpacas is controlled by a non-synonymous SNP in the KIT gene. Alpaca breeders will now be able to make informed choices about planned matings.



# Developing an eDNA Toolkit to Detect the Data-deficient Western Australian Seahorse (*Hippocampus subelongatus*)

Georgia Nester[1], Professor Michael Bunce[2], Dr. Maarten De Brauwer[3], Dr. Joseph DiBattista[4]

[1]Curtin University, [2]Curtin University, [3]Curtin University, [4]Curtin University and Australian Museum.

Environmental DNA (eDNA) is a novel method that uses high-throughput sequencing to detect trace amounts of DNA in the water. It has been used successfully to detect rare sawfish species in Eastern Australia, but has not yet been tested for Hippocampus species, which are significantly smaller than sawfish. The Western Australian seahorse (*Hippocampus subelongatus*) is a charismatic data-deficient species endemic to the temperate waters of Western Australia. Limited data indicates the population is declining in the Swan River, however there are currently no available population estimates. The purpose of this study is to develop an eDNA toolkit to detect the presence of *Hippocampus* species in the marine environment. In this study, primer sets (4) were developed and compared to the fish-specific 16S mitochondrial DNA primers and MiFish primers. Using these primer sets, eDNA metabarcoding sequences were generated from water and sediment samples from various locations across the Perth region of Western Australia. Traditional fish primers (fish-specific 16S and MiFish) failed to detect *H. subelongatus*, whilst the developed primers did to varying degrees of success. This research critically evaluates the role that eDNA surveys can play in detecting cryptic marine biota in Western Australia, in particular the endemic and data deficient Western Australian Seahorse (*Hippocampus subelongatus*).

# Assessing Terrestrial Metabarcoding of Multiple Substrates for Biological Auditing

M. van der Heyde.[1,2], Bunce, M.[2], Wardell-Johnson, G.[1], White, N.[2], Nevill, P.[1]
[1]ARC Centre for Mine Site Restoration, School of Molecular and Life Sciences, Curtin University, [2]Trace and Environmental DNA Laboratory, School of Life and Molecular Sciences, Curtin University

Biological surveys are challenging, expensive, and time consuming, yet crucial for both biodiversity conservation and ecological restoration. Metabarcoding is a disruptive technology that can enable biological auditing from DNA in the environment, and may provide cost-effective monitoring, which can detect flora, fauna and microbial communities. Metabarcoding involves the use of next generation sequencing to sequence barcode regions of the genome to determine the community composition of a sample. These samples can be soil, scat, arthropods, and plant material. This study aims to test multiple substrates (soil, ant middens, scat, plant material, arthropods in pitfall and vane traps) to determine what organisms can be detected from each and where they overlap, as well as how many samples of each may be necessary. Samples were collected in the Pilbara and Swan Coastal Plain region of Western Australia and transported to facilities in Perth where the DNA was extracted, amplified and sequenced using multiple primers and targeting multiple gene regions. Preliminary results indicate that soil samples, despite showing promise for biological auditing in some regions, yields very little plant or animal DNA. While soil samples are necessary to determine soil microbial communities, they may be less able to represent flora and fauna communities. Likely, this is a result of the high temperatures and UV radiation of these Western Australian regions, both of which degrade DNA. Bulk samples, such as arthropods from pitfall traps and vane traps, show far greater promise as DNA is extracted directly from homogenized arthropod samples. The goal of this study is to provide a guide for terrestrial metabarcoding sample collection to be used for biological surveys, particularly in subtropical and Mediterranean regions.

# Diversity of Antibacterial and Physicochemical Properties amongst Monofloral Honeys from Western Australia

Kathryn J. Green [1], Edith Tang[2], Connie Locher[2], Katherine A. Hammer[1]

[1] School of Biomedical Sciences, The University of Western Australia, [2] School of Allied Health, The University of Western Australia

**Introduction**. The aim of this research was to quantify the antibacterial activity of a broad range of Western Australian honeys and to correlate activity with physicochemical parameters. **Problem Statement**. The antibacterial activity of Western Australian honeys is poorly understood and not widely documented, and this study aims to address this. **Procedures**. Fifty honeys from diverse floral sources and from across Western Australia were obtained. Antibacterial activity was investigated by determining the minimum inhibitory concentrations (MICs) of honey against *Staphylococcus aureus* ATCC 29213*, Escherichia coli* ATCC 25922*, Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. Antibacterial activity was also determined by the agar-based Phenol Equivalence Assay. Physicochemical parameters including pH, colour, total phenolic content and hydrogen peroxide generation were quantified. **Results**. A range of antibacterial activity was observed. The five honeys with the highest antibacterial activity were derived from Whitegum (*Eucalyptus wandoo*, n=2), Jarrah (*Eucalyptus marginata*, n=2) and Redgum (*Corymbia calophylla*, n=1). These honeys showed MICs of 3 – 5% (w/v) against *S. aureus*. The least active honey (Spring honey) had MICs of greater 30% (w/v), which was the highest test concentration, against all organisms. Antibacterial activity determined by the Phenol Equivalence Assay ranged from 0 to 53.8 equivalent phenol concentration. The pH range observed was 3.99 - 5.15, and colour ranged from 68.2 to 716.0 mAU. Total phenolic content expressed as gallic acid equivalent, ranged from 49.9 to 121.7 mg/100g and the maximum level of hydrogen peroxide generated ranged from 0 to 479.4 µM. There was no obvious relationship between bioactivity and any single physicochemical parameter. **Conclusions**. This research demonstrates that Western Australian honeys have diverse levels of antibacterial activity and a range of physicochemical characteristics. This data will assist the local honey industry in identifying honeys with high bioactivity and therefore the greatest potential for medicinal use.



# Replicating Clinical Radiation Therapy Protocols in Preclinical Brain Tumour Models

Hilary Hii[1], Brooke Strowger[1], Mathew Ancliffe[1], Tracy Seymour[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**. Surgical resection and targeted radiotherapy, often followed by chemotherapy, have been the foundations of paediatric brain cancer treatment for decades. For brain cancers that often spread throughout the brain and spinal cord, craniospinal irradiation represents the standard-of-care. Animal models that accurately recapitulate the genotypes and phenotypes of paediatric brain cancers are essential to evaluate potential new approaches to therapy ahead of clinical trial. The XRAD image-guided small animal radiotherapy (SmART) system combines CT imaging with precision irradiation using x-rays. **Problem Statement**. Our aim was to optimise preclinical radiotherapy protocols in mouse models of medulloblastoma, ependymoma and glioblastoma. To evaluate the accuracy and efficacy of the device, radiation was administered to a transgenic mouse model of medulloblastoma and to mice with orthotopically-implanted brain tumours. **Procedures**. Acute effects of targeted radiotherapy was analysed by delivering one dose of 2 Gy irradiation and harvesting tissue either 2 or 24 hours post-irradiation. Markers of DNA damage and apoptosis were used to evaluate tumour targeting. In order to model multifractionated radiotherapy, we have trialed 16, 18 and 20 Gy multifractionated radiotherapy, delivering 2 Gy per treatment day. **Results**. A transgenic model of SHH-subtype medulloblastoma (NeuroD2::SmoA1) demonstrated exquisite sensitivity to irradiation with significant DNA damage and apoptosis two hours post-treatment ($p < 0.0001$ and $p = 0.0025$ respectively). Moreover, reduced tumour size was evident by MRI after five days of radiotherapy. In our multifractionated radiotherapy survival studies, multiple models of Group 3 medulloblastoma demonstrated radiation sensitivity and anti-tumour effects were also observed in ependymoma and glioblastoma xenografts. **Conclusions**. These data serve as baseline information for future experimental protocols that will evaluate combinatorial regimens of radiotherapy, conventional chemotherapy, and novel anti-cancer agents. These experiments enable rigorous evaluation and selection of viable agents to consider for future clinical evaluation.

# Application of a Predictive Fibrosis Gene Signature to Liver Steatosis.

[1,2]Priya Sarma, [1,2]Jose Caparros-Martin, [1,2]Fergal O'Gara, [1,2]Nina Tirnitz-Parker, [1,2]Jully Gogoi-Tiwari, [1,2]Gregory Miller, [1]Jeff Hughes, [1,2]Ricky R Lareu;

[1]Pharmacy and Biomedical Sciences and [2]CHIRI, Curtin University; [3]Biomedical Sciences, University of Queensland and Envoi Pathology

**Introduction**. In the wake of urbanisation, metabolic syndrome (MS) is an escalating public-health and clinical challenge worldwide. MS is defined as a combination of metabolic and medical disorders such as obesity, dyslipidaemia, high blood pressure and elevated fasting glucose. They are all risk factors for non-alcoholic fatty liver disease (NAFLD): an accumulation of excess lipids in the liver (steatosis). NAFLD may lead to inflammation and fibrosis, called non-alcoholic steatohepatitis (NASH) and further to cirrhosis and end-stage liver disease. Not all patients with NAFLD progress to more severe liver disease therefore a clinical challenge is to predict which patients will at an early stage. **Procedures**.We applied a recently published gene signature demonstrated to predict progression of NAFLD to NASH, to a pregnane X receptor Knock-Out (PXR-KO), diet-induced obesity model. Mice (Control, C57BL/6J; PXR-KO) were fed a high-fat diet (HFD) for 3 months with/without atorvastatin. Liver histology was evaluated for steatosis, inflammation and fibrosis and liver tissue was used to assess gene expression with quantitative-RT-PCR. **Results.** As expected, steatosis was present in all HFD-fed treatment groups to comparable levels and no fibrosis was detected. Only the treatment groups with atorvastatin demonstrated minor inflammation. We have presently tested 9 (SERPINE1, COL1A1, COL3A1, TNC, TLR4, THBS1, IL1RN, CCL2, CCR2) of the 20 genes from the published predictive signature and all 9 genes were over-expressed in the PXR-KO, with several more highly expressed in the atorvastatin-treated groups. Interestingly, SERPINE1, a serine protease inhibitor, which possessed the highest composite score in the gene signature, was highly expressed in both the atorvastatin and vehicle PXR-KO groups as well as the control group with atorvastatin. **Conclusions**. Although fibrosis was not detected histologically, applying this gene signature provides further information as to the activated underlining molecular mechanisms that identify which treatments are potentially more severe and may lead to fibrosis.



# A Role for Early Oral Exposure to House Dust Mite Proteases in Food Allergy Susceptibility

Akila Rekima[1], Patricia Macchiaverni[1], Chrystelle Bonnart[2], Jessica Metcalfe[3], Meri Tulic[4], Nicolas Halloin[4], Jon Genuneit[5], Samantha Zanelli[4], Samara Medeiros[6], Debbie Palmer[3], Susan Prescott[3], Valerie Verhasselt[1].

[1]University of Western Australia. [2]INSERM U1220, France. [3]Telethon Kids Institute, Australia. [4]Universite de Nice Sophia Antipolis, France. [5]Ulm University, Germany. [6]UFMG, Brazil.

We recently demonstrated that allergens from house dust mite *Der p* are present in human milk and thereby could potentially affect gut mucosal immunity in offspring. Here, we observed that mice exposed to *Der p* allergens through breastmilk showed increased gut permeability, IL-33 secretion, group 2 innate lymphoid cells activation and Th2 cell differentiation in the small intestine at 2 weeks of age. This pro-Th2 gut mucosal environment hindered the induction of antigen-specific FoxP3 regulatory T cells upon oral exposure to egg-derived antigen ovalbumin (OVA) through breastmilk. In the long term, the *Der p*-induced imbalance in gut mucosal immunity abolished the possibility to prevent food allergy by OVA exposure through breastmilk. Neutralization of *Der p* protease activity indicated that this enzymatic activity was necessary and sufficient for Der p-induced gut mucosal immune dysregulation and increased food allergy susceptibility. Finally, we observed a consistent relationship in humans. In a birth cohort of 100 infants, IgE-mediated egg allergy prevalence at one year was 4.7 times higher in infants exposed to *Der p* through breastmilk compared to infant exposed to OVA through breastmilk. The proteases from house dust mite could profoundly affect gut immune ontogeny and the risk for food allergy, should promote research for new strategies to prevent food allergy in early life.

# Colostrum is Necessary for Type 2 Immunity Development in Neonates

Akila Rekima[1], Charlotte Isnard[2], Danielle J. Smyth[3], Rick M. Maizel[3], Valérie Verhasselt[1,4]

[1]School of Molecular Sciences, University of Western Australia, Perth, Australia, [2]Neonatology / department of paediatrics Hopital de l'Archet II, France, [3]Institute of Infection and Immunology Research and Centre for Immunity, Infection and 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.



# Considerations and Case Studies in the Design and Implementation of eDNA Biosecurity Surveillance using Metabarcoding

Tiffany Simpson[1], Adam Koziol[1], Tina Berry[1], Michael Stat[2], Justin McDonald[3], et al, Mike Bunce[1],

[1]Trace and Environmental DNA Laboratory, Curtin University, Australia. [2]Maquarie University, Australia, [3]Department of Primary Industries and Regional Development, Australia

Environmental DNA (eDNA) metabarcoding using high throughput sequencing (HTS) is rapidly becoming a valuable biosecurity tool in marine surveillance. While these technologies are powerful for the accurate and efficient detection of species within complex biological samples, optimising sampling design and robust eDNA workflows are critical in producing meaningful results. Through a variety of studies, we examined factors such as seasonality, substrate selection, DNA movement, replication and sensitivity. We found that these influences should be carefully considered when determining the sampling design, as they are not only important in the successful establishment of invasive species but can also significantly impact the potential for detection. Using the findings of these studies we aim to develop best-practice eDNA strategies across a number of applications including; invasive marine species (IMS) detection, establishment of baselines, in-water vessel cleaning and ballast water treatment. The results of these studies will be presented in the context of the commercial services, focusing on IMS detection and surveillance, which we are now able to provide to industry partners.

# Combining Chk1/2 Inhibition with Radiation Enhances *in vitro* and *in vivo* Cytotoxicity in Group 3 Medulloblastoma

Brooke Strowger[1], Meegan Howlett[1], Hilary Hii[1], Mani Kuchibotla[1], Patrick Dyer[1], Raelene Endersby[1] and Nicholas Gottardo[1,2].

[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.

Medulloblastoma is a heterogeneous disease, comprising four molecular subgroups, termed WNT, SHH, Group 3 and Group 4. Each has distinct clinical, pathological, and molecular features. Surgical resection and craniospinal irradiation followed by chemotherapy are the mainstay of medulloblastoma treatment. Despite treatment intensification, survival has plateaued for the past two decades at around 70%. Certain sub-groups, namely those with SHH with p53 mutations and Group 3 with *MYC* amplification, have dismal prognoses. Since medulloblastoma patients that relapse are essentially incurable, ergo, the best opportunity to cure medulloblastoma is during frontline therapy. To increase cure rates, we employed an unbiased high-throughput drug screening approach, to identify novel drugs that enhance the effects of current treatments to aid front-line therapy. We identified prexasertib, an inhibitor of the cell cycle checkpoint kinases 1 and 2 (Chk1/2), as a promising candidate. Chk1/2 are critical regulators of the DNA-damage response. Given radiation is a potent inducer of DNA damage, we hypothesised that Chk1/2 inhibition with prexasertib may enhance radiation-induced cytotoxicity in medulloblastoma. In this study, we found that radiation induced a strong DNA damage response in multiple medulloblastoma models. *In vitro*, prexasertib reduced the colony-forming ability of medulloblastoma cells post-irradiation. *In vivo*, tumour-targeted radiation therapy and prexasertib each induced modest apoptosis in orthotopically-implanted medulloblastomas, which was significantly increased when co-administered. Importantly, this combination therapy also improved the survival of mice with medulloblastoma. Taken together, these data reveal significant anti-tumour effects when radiation is combined with Chk1/2 inhibition suggesting that this combination therapy may increase treatment efficacy.



# Enhancing Responses to Cancer Vaccines with Novel Adjuvants

Teagan Wagner[1], Clara Ta[1], Katherine Audsley[1], Anthony Buzzai[1], Bree Foley[1], Jason Waithman[1]

[1]Cancer Immunotherapy Unit, Telethon Kids Institute, University of Western Australia

Immunotherapies are revolutionizing cancer treatment, but further advances are required to ensure their success in the majority of patients. There are multiple approaches to harnessing the immune system to fight cancer. Our research focuses on anti-cancer vaccines and their ability to force $CD8^+$ killer T cell immunity. An important component of vaccine design is the adjuvant, which is incorporated to 'boost' immunity against cancer. We asked whether members of the Type I Interferon (IFN) family, cytokines known widely for their potent immunomodulatory capacity, could enhance tumour-specific $CD8^+$ T cells when incorporated as adjuvants into a whole cellanti-cancer vaccine. To explore this possibility, we actively immunised mice with irradiated tumour cells genetically engineered to secrete a single IFN subtype. To date, we have tested 7 of the 15 IFN subtypes and our results show each subtype consistently increases the expansion of tumour-specific $CD8^+$ T cells as compared to vaccine alone. A superior adjuvant candidate was identified, $IFN_Z$, as the IFN subtype that produced the greatest expansion performing significantly better than one of the gold standard adjuvants used in current cancer vaccine strategies, poly I:C. Incorporating $IFN_Z$ into a therapeutic vaccination protocol enhanced cancer immunosurveillance, and resulted in the complete eradication of tumour formation in mice bearing melanomas. Strikingly, the same tumour-free mice remained resistant to re-challenge with the same cancer cells more than 60 days later, demonstrating robust immunological memory. This data provides strong rationale for incorporating $IFN_Z$ as a natural adjuvant into future vaccine protocols. Furthermore, the power of $IFN_Z$ to remarkably improve anti-tumour immunity could have significant implications in other immunotherapies and ultimately lead to greater success rates.

# Antisense Oligonucleotide Treatment of *COL7A1* Exon 73 Causes Non-specific Pre-mRNA Processing

Kristin A West[1], Sue Fletcher[1,2], Steve D Wilton[1,2]

[1] Centre for Comparative Genomics, Murdoch University, [2] Perron Institute for Neurological and Translational Science

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare inherited disease caused by mutations in *COL7A1*, which encodes the type VII collagen. This protein forms the anchoring homotrimer filaments that strengthen and stabilise the epidermal basement membrane layers in skin. Symptoms of RDEB include severe blistering of the skin and mucous membranes. A high number of mutations that cause RDEB cluster in exon 73, frequently amino acid substitutions or premature termination of translation that both substantially affect protein function and stability. Whilst developing and optimising a novel therapeutic strategy to treat RDEB using antisense oligonucleotides (AOs) to remove exon 73 from the mature mRNA transcript, non-specific pre-mRNA processing of neighbouring exons and introns was observed. Although removal of exon 73 was possible, several AOs not only promoted the removal of exons 73 and 74 but also caused inclusion of intron 76, activating an in-frame premature termination codon found within the intron. Subtle modifications of AO design targeting this region of exon 73 lead to various splicing patterns and ratios of exon skipping and intron inclusion. To explore the underlying mechanism of this phenomenon, we examined the targeted splicing enhancer in more depth, and investigated the sequence of intron removal from the pre-mRNA transcript in the region, thereby providing more insight to the splicing process and assist in improved AO development.