Poster Presentations

STUDENT POSTERS	POSTER SESSION	LOCATION
Agricultural Biology & Plant Science	A	Lower Colonnades
Biochemistry and Molecular Biology	B	
RNA Biology	C	
Cell and Developmental Biology	D	
eDNA and Environmental Genomics	E	
Cancer Biology (Health Sciences)	F	Upper Foyer
Immunology and Inflammation	G	
Microbiology	H	Upper Colonnades
Neuromuscular and Neuroscience	I	
ALL OTHER SCIENTISTS		
Biomedical Science	J	Seminar Room 3
Immunology	K	(upper level)
Agricultural Biology & Plant Science	L	
Marine Science	M	

Poster Presentations

STUDENT POSTERS		ALL OTHER POSTERS			
Alkresheh RO	B4	Iluoreh AH	D4	Bagherian M	L2
Auzins R	F6	Inder-Smith K	B1	Chooi H	L4
Ayad ME	E1	Jawahar MJ	C5	Clifton BE	M5
Batohi N	G1	Jayasankar HS	F5	Debler J	L1
Bertazzo H	I2	Lee PKH	D2	Fang C	L6
Bhalla N	B5	Lighton M	G4	Faseeh I	M4
Bhatti S	B3	Lim A	F3	Fraser M	M2
Boulter J	G3	Lines G	H2	Hii H	J2
Chnadrsekaran KN	C7	Link S	A8	Huynh DS	M3
Coles Y	A5	Morikawa S	A7	Kuek V	J5
Davis J	E5	O'Keeffe A	I1	Larcher L	J3
Depiazzi A	E2	Perera HSL	I4	Morici M	K5
Dey S	F8	Pirotta D	I3	Phan H	L5
Dixon A	C3	Purbrick HG	G8	Pitout I	J1
Dymock S	F1	Rhodes X	A2	Raes EJ	M1
Dyton A	F4	Russell H	D3	Stevens K	K1
Faber A	B7	Saengruang N	B6	Takahashi A	J4
Flanagan C	D1	Sahchithananthan S	C4	Takahashi A	K2
Flint R	H1	Saville S	C1	Verdonk C	L3
Gao C	C6	Sbrana L	H6	Warburton P	K3
Gomes AR	G7	Schreurs J	G2	Zaenker P	K4
Gomez P	D5	Sexauer D	F7		
Gray N	A1	Slater N	I5		
Guthrie A	E3	Szabo D	H5		
Han V-C	E4	Tau M	G5		
Handley D	G6	Tenaglia V	H4		
Hapuarachchige R	A6	Tony AK	A4		
Heendeniya S	B2	Wang A	F2		
Henderson-Kelly Y	D7	Worth L	C2		
Hossain M	A3	Wright OK	D6		
Hyatt BL	H3				

Comparative Pan-Genomics of Multiple Crop Pathogen Species to Study Virulence Genes

Naomi Gray[1], Mohitul Hossain[1], Pavel Misiun[1], Johannes Debler[1], Bernadette Hernares[1], Manisha Shankar[2], Hossain Golzar[2], Elzette Palmiero[1], Simon Elwood[1], Kristina Gagalova[1,3], Pao Theen See[1], James Hane[1]

[1]Centre for Crop Disease Management (CCDM), Curtin University, [2]Department of Primary Industries & Regional Development, [3]Analytics for the Australian Grains Industry (AAGI)

Effective prediction of fungal effector genes is an important aspect of crop disease management and surveillance. Our recently developed bioinformatics pipeline designed to mine fungal pan-genomes for pathogenicity data called MycoProcessor (with the tool EffectorFisher), can predict effector-like candidates with cultivar-specific association. This requires corresponding sequencing and phenotype data for large numbers of isolates which is not currently available for most species. However, with the quantity of fungal genome sequencing currently available we have an opportunity to use comparative pan-genomics to identify effectors and other virulence genes with broader specificity at the host level. Our dataset contains a range of pathogens that include closely related species with differing host ranges and pathogenic lifestyles. These include: Pyrenophora spp. (P. tritici-repentis (n=112), P. teres f. sp. teres (n=401) and P. teres f. sp. maculata (n=57)) and Parastagonospora nodorum (n=650), collectively infecting wheat and barley hosts; Ascochyta fabae (n=70) and A. lentis (n=150) infecting faba bean and lentil, respectively; Rhizoctonia solani AG8 (n=43) infecting wheat and lupin; Colletotrichum lupini (n=44) and Diaporthe toxica (n=36) infecting lupin. In addition to predicting effectors, we can also mine fungicide-resistance mutations and examine rates of repeat-induced point mutation, lateral gene transfer, and mesosynteny across this diverse set of pan-genomes.

Xavier Rhodes
Curtin Unviversity

Introduction: Cryopreservation is a valuable long-term storage method for plant germplasm. While successful cryopreservation protocols have been established, challenges can arise in the development of cryo-protocols for new species. Consequently, there is a need to understand aspects of plant physiologies relevant to cryopreservation. Mitochondrial function is fundamental to metabolic activity and is believed to play a crucial role in adapting to the stressors of cryopreservation. **Problem statement**: It is not well understood how cryoprotective agents (CPAs) and their use in plant vitrification solutions (PVS) affect mitochondrial function. **Procedures:** Mitochondrial function under the exposure of various CPA treatments was investigated using extracellular flux analysis (XF). **Results**: It was demonstrated that parameters of mitochondrial function were not significantly impacted by exposure to dilute 1% PVS2 (composed of glycerol, ethylene glycol (EG), dimethyl sulfoxide (DMSO) and sucrose), while 10, 20, 50 and 100% PVS2 did have significant impacts on various parameters of mitochondrial function. Exposure to DMSO and EG caused significant declines in spare respiratory capacity and elevated alternative oxidase (AOX) activity. The addition of sucrose proved beneficial to mitigate the decline in spare respiratory capacity due to the combined action of DMSO, EG and glycerol. Across various mitochondrial function parameters, the ratio of DMSO, EG, glycerol and sucrose in PVS2 appeared to partially mitigate the deleterious effects seen under the exposure to the individual CPAs and varied CPA ratios. **Conclusions**: It was thus demonstrated that while PVS2 and its CPA components were deleterious to mitochondrial function, the combination of CPAs used in PVS2 can mitigate such effects.

Improving fungal effector prediction and enabling resistant cultivar selection by integrating disease phenotyping and pathogen pan-genomics

Mohitul Hossain[1], Kristina Gagalova[1,2], Naomi Gray[1], Pavel Misiun[1], Fiona Kamphuis[1], Lukas Hunziker[1], Eiko Furuki[1], Hossein Golzar[3], Manisha Shankar[3], Huyen Phan[1], James Hane[1]

[1]Centre for Crop and Disease Management (CCDM), Curtin University [2]Analytics for the Australian Grain Industry (AAGI), Curtin University [3]Department of Primary Industries and Regional Development (DPIRD)

Fungal effectors interact with host cognate receptors to cause disease, and their accurate identification is important for crop disease management. In the last decade, effector prediction tools based on physicochemical properties have significantly improved in accuracy. However, a gap remains between the number of predictions and feasible experimental validation, so obtaining a reliably reduced candidate list is important. EffectorFisher is a novel approach integrating protein isoform profiles derived from pathogen pan-genomic data with disease phenotyping to narrow down effector candidates. It was tested on the *Parastagonospora nodorum*-wheat (necrotrophic) and *Zymoseptoria tritici*-wheat (hemibiotrophic) pathosystems. It improved the ranking of known effectors by 2-13 times, reduced candidate numbers by ~3.5-fold, and predicted cultivar-specific effector interactions for both necrotroph (inverse gene-for-gene) and hemibiotroph (gene-for-gene) pathosystems. Minimum-viable experimental design was assessed using simulated datasets with reduced numbers of *P. nodorum* isolates and/or phenotyped cultivars, which generated comparable results for known effectors for upwards of ~40 isolates and ~4 cultivars. It also outperformed GWAS, and can provide results with non-quantitative phenotyping (i.e. cultivar-of-isolation). EffectorFisher also led to development of a new method, CultSelect, designed to predict the disease phenotype of cultivars from new pathogen sequence data.

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Dissecting the Australian Wheat Powdery Mildew Pathotypes

Ancy Kuthokathan Tony[1,3], Daniel Mullan[2], Huyen Phan[1,3], Fran Lopez Ruiz[1,3], Ben Saunders[3], Simon Ellwood[1,3] & Kar-Chun Tan[1,3*]

[1]The Centre for Crop and Disease Management, [2]Intergrain Pty Ltd, Bibra Lake, Australia, [3]School of Molecular and Life Sciences, Curtin University, Bentley, Australia. *Kar-Chun.Tan@curtin.edu.au

Blumeria graminis f. sp. *tritici*, now known as *B. graminis*, the causal agent of wheat powdery mildew, is a significant threat to wheat production in Australia. The widespread susceptibility of commonly grown cultivars has increased dependence on fungicides, which can accelerate the development of fungicide-resistant pathogen strains. Genetic resistance offers a sustainable alternative for disease management, but its strategic deployment requires a clear understanding of the pathogen’s population structure and pathotype diversity. This study addressed the need to identify effective resistance (*R*) genes against contemporary Australian *B. graminis* populations and to understand their geographic variation in virulence. A total of 30 *B. graminis* isolates collected between 2020 and 2024 from various Australian wheat-growing regions were evaluated on 24 wheat lines carrying defined *R* genes. Disease responses were recorded, and clustering analysis was used to identify pathotype groups based on virulence profiles. Clustering revealed multiple distinct pathotypes within the Australian population of *B. graminis*. The *R* genes, such as *Pm4a*+ and *Pm2*+, with unknown genetic resistance, showed resistance to all isolates tested. *Pm16*, *Pm3a, Pm17, Pm4a*, and *Pm4b* were partially effective against most Eastern and Western Australian isolates. The study achieved its objective of identifying effective *R* genes and revealed regional differences in pathotype virulence. These findings provide valuable guidance for breeding programs to prioritise and deploy *R* genes that offer durable and broad-spectrum resistance against wheat powdery mildew in Australia.

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Discovery of *Starship* Large Transposons and Transposon Expansion in *Pyrenophora teres f. teres* Associated with Increased Virulence in Barley

Yutathkarn Coles[1], Ciara Gifford[1], Sabrina Cuellar[1], Lilian Sanglard[1], Robabeh Ghavamabad[1], Kul Adhikari[1], Kithsiri Jayasena[2], Johannes Debler[1], Ayalsew Zerihun[1], Mark Gibberd[1], Fatima Naim[1]*

[1]Centre for Crop and Disease Management, Curtin University, [2]DPIRD

Australian barley production yields $2.7 billion annually, reduced by disease impacts stemming from Net Form Net Blotch. This disease is caused by necrotrophic fungal pathogen Pyrenophora teres f. teres (Ptt), which has demonstrated recent host adaptation to commercial elite barley. We investigated the evolution and genetic basis of virulence in eight new isolates of Ptt, collected from WA elite barley, 2019-2023. Disease phenotyping confirmed increasing virulence on malting barley and the emergence of aggressively virulent pathotypes. High molecular weight DNA extraction, long-read sequencing via Oxford Nanopore Technologies and in-house assembly and annotation yielded eight high-quality, comprehensively annotated Ptt genomes. Multiple alignment to the previous Beecher virulent pathotype assembly revealed rapid, localised divergence at expanded repeat-rich regions, which were associated with isolate-specific genes matching secreted fungal effector characteristics. Large mobile elements were identified with the tool Starfish, isolates were combined with 290 Dothideomycete genomes from NCBI and JGI to uncover five candidate Starships spanning multiple haplotypes. These different Starships are present globally and only within Australian sub-clades, encoding complex genomic rearrangements and internal nested transposon insertions. One Serenity-class Starship was identified in a Canadian isolate of sister species Pyrenophora tritici-repentis, causing Yellow Spot in wheat. Another unclassified Starship was found conserved in global isolates and wild Western Australian barley-grass infecting isolates. Given the high divergence within Starship haplotypes and their distribution, we identify Starships as active drivers of diversifying evolution in fungal pathogens populations, and markedly unique between newly-sequenced Western Australian isolates. Additionally, we speculate that Starships contribute to de novo emergence and horizontal transfer of effectors between fungal pathogen populations, resulting in the emergence of virulent Ptt strains. This study contributes to the growing body of evidence that Starships drive rapid fungal pathogen evolution, and develops genomic resources required for investigating molecular mechanisms of disease in NFNB.

A study on elemental composition and phosphorous dissolution of steel slag waste for its' potential use in agriculture

Ruwani Hapuarachchige[1], Nanthi Bolan[1], Kadambot Siddique[2], Zakaria Solaiman[1], James O'Connor[1]

[1]School of Agriculture and Environment, UWA, [2]Institute of Agriculture, UWA.

Efficient nutrient management, particularly phosphorus (P), is critical to achieve sustainable agricultural practice. The finite nature of phosphate rock and the environmental impacts of conventional P fertilisers highlight the need for alternative nutrient sources. Steel slag, a by-product of steelmaking, contains P and other plant nutrients offering a potential recycling pathway for industrial waste. There is growing research interest in techniques for removing the P-rich phase from steel slag, as excess P reduces steel quality. Isolating and removing this phase would allow the slag to be recycled in steel production. However, challenges remain in optimising P extraction, the understanding of nutrient release in different soils, and ensuring environmental safety. This study analysed the chemical characteristics of the Basic Oxygen Furnace Slag (BOFS) alongside Synthetic Slag (SS), which was prepared under lab conditions to simulate BOFS for comparative study purposes. Samples were analysed for pH and elemental composition using fusion digestion followed by ICP-OES, and P dissolution was assessed using Neutral Ammonium Citrate (NAC) and 2% Formic Acid (FA) extractions. In the FA extraction method, P dissolution was compared to tri-calcium phosphate, struvite and Triple Super Phosphate (TSP). BOFS and SS were highly alkaline, indicating the liming potential. Chemical analysis confirmed that steel slag contains macro- and micronutrients such as calcium, iron, and magnesium, iron, manganese and zinc with detectable levels of heavy metals, including chromium and vanadium. Total P content was 1% for BOFS and 3% for SS, markedly lower than TSP. FA extractability as a percentage of total P was also significantly lower in BOFS (41%) and SS (33%) than TSP (99%). P extractability of steel slag materials with NAC was greater than FA extractability. These results indicate the potential for recycling steel slag waste as in agriculture, demanding further studies to focus on separating P rich steel slag, plant availability and evaluating environmental safety. "This research was supported by BHP INNOVATION PTY LTD. The company had no role in analysing the data or preparing the abstract."*

Compensatory Mechanism of Virulence Factors in a Fungal Pathogen of Wheat

Shota Morikawa[1], Callum Verdonk[1], Johannes Debler[1], Huyen Phan[1], Chala Turo[1], Christina Grimes[1], Kasia Clarke[1], Darcy Jones[1], Bernadette Henares[1], Evan John[2], Carl Mousley[3] & Kar-Chun Tan[1]

[1]Centre for Crop and Disease Management, [2]University of Bonn, [3]Curtin Medical Research Institute

The fungal pathogen Parastagonospora nodorum infects wheat by secreting small proteins called necrotrophic effectors. A P. nodorum mutant (toxa13) with the deletion of three major necrotrophic effector genes was still able to cause infection in various wheat lines comparable to wildtype despite lacking three major virulence genes. In this study, a transcriptomics analysis found that the previously characterised effector genes and putative effector genes have been up-regulated in the toxa13 mutant compared to the wildtype strain during infection of the host. Characterisation of the up-regulated putative effector genes resulted in the discovery of 15 novel necrosis inducing non-host effectors of P. nodorum. The mechanism may be conserved across different phytopathogens and therefore the method used in this study could be applied to discover novel possible virulence factors in other fungi.

Optimising genetic resistance to barley spot form net blotch through mutagenesis

Sundara Link[1], Simon Ellwood[1], Pao Theen See[1] & Hoan Hinh[1]

[1]Curtin University, Centre for Crop and Disease Management

Spot form net blotch (SFNB), caused by *Pyrenophora teres* f. *maculata* (PTM), is a significant disease of barley in Australia, leading to substantial yield losses and economic impact. Due to the recent emergence of SFNB, limited natural resistance exists within current barley germplasm. There is an urgent need to develop resistant cultivars and to improve our understanding of PTM pathogenicity. This research addresses two major challenges: (1) the lack of genetic resistance to SFNB in barley breeding lines, and (2) the limited understanding of the fungal genetic mechanisms underlying PTM virulence. To uncover novel sources of host resistance, chemically mutagenized barley populations were screened using a virulent PTM isolate. Phenotypic responses can then be evaluated and followed by sequencing, crossing, and gene mapping. In parallel, a large-scale Genome-Wide Association Study (GWAS) was performed on diverse barley cultivars under field conditions, using phenotypic data and whole-genome sequencing to detect significant SNPs and candidate resistance genes. To investigate pathogen genetics, PTM isolates were subjected to ultraviolet (UVC) mutagenesis to induce random mutations. Changes in virulence can then be assessed through infection assays and confocal microscopy using dual-staining (WGA-FITC and propidium iodide) to visualize fungal colonization and plant tissue responses. Mutants displaying altered pathogenicity can then undergo whole-genome sequencing, chromosomal mapping, transcriptome analysis, and pathotype profiling. Mutant barley lines exhibiting altered resistance were identified, and GWAS analyses revealed genomic regions associated with SFNB resistance. UVC-induced PTM mutants showed altered infection phenotypes, allowing for the correlation with genetic changes in candidate virulence genes and regions. This dual host-pathogen strategy successfully identified novel resistance loci and fungal virulence factors. These findings contribute valuable genetic resources for barley breeding and advance our understanding of PTM–host interactions, supporting the development of durable SFNB resistance.

This research was supported by GRDC (Grains Research & Development Company) & WAARC (WA Agricultural Research Collaboration). Neither company had a role in analysing the data or preparing the abstract.

Iron-Mediated Lipid Alterations in Live Pancreatic Beta Cells Revealed by Infrared Spectroscopy

Keea R Inder-Smith[1,2], Cyril DS Mamotte[1,2], Clinton J Kidman[3], Annaleise R Klein[4], Jitraporn Vongsvivut[4], Mark J Tobin[4], Mark J Hackett[2,5], and Ross M Graham[1,2]

[1] Curtin Medical School, Western Australia; [2] Curtin Medical Research Institute, Western Australia; [3]Department of Geology, University of Saskatchewan, Canada; [4] IRM Beamline, Australian Synchrotron, Victoria; [5] School of Molecular and Life Sciences, Curtin University, Western Australia

Obesity is positively associated with lipid infiltration into pancreatic tissue, which may lead to non-alcoholic fatty pancreas disease (NAFPD), the pancreatic manifestation of metabolic syndrome. Dysregulated iron metabolism not only has the potential to cause tissue damage, but is also hypothesised to cause fatty replacement of pancreatic tissue. This, in turn, may lead to NAFPD, progression to steatopancreatitis and possible pancreatic failure. Nevertheless, research into NAFPD is still in its infancy and its clinical significance and underlying biochemistry are not well understood. BRIN-BD11 cells, a pancreatic beta cell line, were seeded on to CaF_2 discs and incubated for 8 hours in control or high iron experimental media. Single live cells were then imaged for a further period of 8 hours in a modified FTIR flow cell at the Australian Synchrotron's mid-IR beamline to assess iron's effect on lipid metabolism. Lipid metabolism was significantly impacted by iron loading. Cells exposed to iron had significantly lower cholesteryl esters and triglycerides across all time points when compared to control cells. Similarly, iron-loaded cells exhibited significantly more free fatty acids compared to controls. Together, these results suggest that iron may be inducing release of fatty acids from triglycerides. Loading cells with iron had no impact on lipid chain length or lipid saturation, suggesting that iron does not induce controlled lipid oxidation. Additionally, lipid parameters remained stable over time between 8 and 15 hours, indicating that iron-associated lipid changes occur relatively quickly and had reached equilibrium by 8 hours. **Conclusion**. We have demonstrated that the iron status of pancreatic beta cells significantly impacts lipid metabolism. Given both iron and lipids are associated with pancreatic disease, examination of the relationship between lipid and iron metabolism will be critical for underpinning better prevention and more effective treatments for NAFPD.

A novel 'seesaw' dual therapy approach to mitigate Alpha-1 Antitrypsin Associated Liver Cytotoxicity

Saumya Heendeniya[1,2], John Chen [1], Bal Hari Podal[1,2] Rakesh N. Veedu[1,2]

[1]Personalised Medicine Center, Murdoch University, [2]Perron Institute fScience, Australia.

Alpha-1 antitrypsin (A1AT) deficiency represents a predominant genetic aetiology of liver disease and transplantation in paediatric populations, with its most severe manifestation occurring in approximately 1 in 3,500 live births. The cytotoxicity associated with alpha-1 antitrypsin is a principal cause of inherited liver disease in both children and adults. This cytotoxicity arises from the aggregation of the alpha-1 antitrypsin protein within hepatocytes, a consequence of the PiZZ mutation in the *SERPINA1* gene. Current experimental therapies are directed towards preserving liver function, with a significant emphasis on enhancing autophagy to degrade mutant A1AT proteins. Genetic therapies, including gene editing and RNA interference, are under active development. This study investigates an innovative "seesaw" therapeutic approach, employing splice-modulating antisense oligonucleotides (ASOs) to downregulate mutated *SERPINA1* expression while utilizing synthetic mRNA of *SERPINA1* to upregulate functional A1AT protein. This seesaw therapeutic strategy integrates two distinct nucleic acid therapeutic techniques to mitigate liver cytotoxicity by downregulating the cytotoxic non-functional A1AT protein in patients while upregulating the functional A1AT protein. ASOs were designed to target mutated *SERPINA1* to disrupt the production of mutant A1AT. It was hypothesized that targeting exons 2, 3, and 4 for skipping would result in a translation arrest. Initially, 14 ASOs were designed, and from preliminary in vitro screening, ASO No. 2 and 3 emerged as leading candidates. ASO No. 2 was shown to target exon 2, leading to the masking of the start codon and potentially arresting translation. Exon 3 skipping was also observed during the investigation, and this dual exon skipping could be advantageous for disrupting A1AT production. Synthetic SERPINA1 mRNA was synthesized, and multiple concentrations (n=3) of the mRNA were used to transfect A1AT deficient patient cells (GM02522) to reestablish the expression of functional A1AT protein. After 24 hours of transfection, 0.625 ng μL^{-1} A1AT mRNA showed a 97.75% (±11.77) increase; 1.25 ng μL^{-1} A1AT mRNA showed a 124.43% (±16.13) increase; and 2.5 ng μL^{-1} A1AT mRNA showed a 177.45% (±25.97) increase in functional A1AT expression ($R^2 = 0.9594$). This novel therapeutic strategy known as seesaw therapy shows promise in reducing liver cytotoxicity while resolving the underlying deficiency of functional A1AT protein.

Fatty Acid Bioconjugation as a Strategy to Enhance Antisense Oligonucleotide Based Therapy

Saadia Bhatti[1,2], Bal Hari Poudel[1,2], Rakesh N Veedu[1,2]

[1]Perron Institute of Neurological and Translational Sciences, [2]Personalised Medicine Centre, Future Health Institutes, Murdoch University

Targeted RNA therapies remain limited by poor cell uptake across lipophilic barriers and off-target distribution. To address this, we investigated fatty acid (FA) bioconjugation as a strategy to enhance antisense oligonucleotide (ASO) uptake, stability, and delivery efficiency in muscle cells. Using fatty acids with longer alkyl chains and cleavable linkers, we tested a panel of FA-ASOs (FA1–FA5) in the H-2Kb-tsA58 mdx muscle disease model. Following pre-screening at 400 and 200 nM concentrations, time-dependent (24–96 h) and dose–response (200 - 25 nM) studies were performed without transfection reagents. The Exon 23 skipping efficiency of FA-ASOs were evaluated by RT-PCR, and nuclear localisation was evaluated using immunofluorescence assay. FA-ASO2 and FA-ASO3 achieved the highest exon skipping at low doses (40% and 56%, respectively), compared to naked ASO control (16%). In contrast, more hydrophobic candidates (FA-ASO4 and FA-ASO5) induced dual exon 22/23 skipping, suggesting that excessive hydrophobicity compromises specificity. These findings also indicated that the other models need to be explored to screen FA-ASO. Ongoing functional studies are focused on clarifying the delivery mechanism, serum stability, cytotoxicity, and immunogenicity of FA-conjugated ASOs across additional *in vitro* models. Our results support FA bioconjugation as a promising strategy to overcome delivery barriers in RNA therapeutics for neuromuscular disease.

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Fibroblasts heterogeneity underlies functional differences between primary and recurrent keloids

Rozan Odeh Alkresheh [1,2,3], Dr Andrew Stevenson, [1,2,3], Dr Nicole Smith, Dr K. Swaminathan Iyer [1,3], Wolfgang Jarolimek [4], Ross Hamilton [4], Dr Fiona Wood [1,2] Dr Mark Fear [1,2,3]

[1] University of Western Australia, [2]Fiona Wood Foundation, [3] ARC Training Centre for Next Gen Technologies in Biomedical Analysis, [4]Syntara

Introduction. Keloids are a type of abnormal scar that occurs after skin trauma. Clinically, keloids are more aggressive and severe than other types of scars. Patients with keloids are more likely to have pain, discomfort, and pruritus than the injured patient. Keloids do not resolve spontaneously and progress over time. Despite a variety of treatment options, keloid recurrence continues to be a significant clinical problem. In these studies, we are investigating how the cellular and molecular biology of keloids changes with recurrence. **Problem Statement**. Research have been focusing on primary keloids; however, recurrent keloids remain under-researched. We hypothesise that cellular changes after treatment might alter the keloid trajectory and may influence the most appropriate treatment. **Procedures/Data/Observations**. We performed fibroblast profiling from primary and recurrent keloids using quantitative polymerase chain reaction (qPCR), immunohistochemistry and migration assays. We also used flow cytometry and cell sorting to identify and isolate fibroblast subtypes within primary and recurrent keloids to assess their potential role in the disease. **Results**. Initial studies showed a decrease in the COL I: COL III ratio in the recurrent keloid fibroblasts compared to the primary keloid fibroblasts. In addition, primary keloid fibroblasts showed faster migration than those from the recurrent keloid. Flow cytometry was conducted using CD26, CD90, CD140a, CD266 and Alpha-SMA cell surface antibodies. CD140a positive fibroblasts were significantly increased in recurrent keloids compared to the primary keloid samples. Further analysis supported that this change in CD140a positive cells was likely underpinning the functional changes identified in Collagen production and migration. **Conclusions**. The studies to date show significant changes in the functional properties of fibroblasts from recurrent and primary keloids. This appears to be linked to changes in specific sub-populations of fibroblasts. Further characterization of these changes may facilitate better targeting of recurrent keloids to improve treatment outcomes.

Optimising DNA Isolation from Ram Semen for Reproductive Applications

Nuttiya Saengruang[1], Mieghan Bruce[1,2], Charlotte Oskam[2,3], Pete Irons[1]

[1] School of Veterinary Medicine, [2] Centre for Biosecurity and One Health, Harry Butler Institute, School of Medical, Molecular and Forensic Sciences, Murdoch University

Sheep production is a multibillion-dollar industry for Australia, supplying both meat and wool. Reproductive biotechnology including sexing semen and embryo transfers play a vital role in improving production. Gamete cell DNA, such as sperm and eggs, become a key focus in sheep reproductive research. Targeting the ram gamete cell, semen provides both billions of sperm cells and contains other cells from the reproductive tissues. Moreover, during the semen collection, they can be contaminated with epithelial cells or leukocytes. This experiment aims to assess which cell lysis method yields the highest DNA copy number while minimising non-sperm cell contamination. Five cell lysis methods were designed to evaluate the copy number of DNA compared to the DNeasy Blood & Tissue kit (control), which is a standard method to extract the DNA from somatic cells. gDNA from each extraction method was quantified by Quantitative Polymerase Chain Reaction (qPCR) using the 18S rRNA gene. The result showed that the method treated with DTT yielded a significantly higher DNA copy number compared to the method without DTT ($P < 0.05$), as DTT facilitates the lysis of the acrosome to release spermatozoa DNA. The semen digest plus DTT method yielded the highest DNA copy number ($35,710,031.12 \pm 7,183,414.16$). These findings indicated that the standard DNA commercial kit (DNeasy Blood & Tissue) can extract spermatozoa DNA by combining with DTT. This optimization could lead to the development of a simple and rapid method for DNA extraction suitable for on-farm usage, offering farmers an accessible approach for applications such as sex selection in the sheep industry.

Optimising DNA Isolation from Ram Semen for Reproductive Applications

Nuttiya Saengruang[1], Mieghan Bruce[1,2], Charlotte Oskam[2,3], Pete Irons[1]

[1] School of Veterinary Medicine, Murdoch University, Murdoch, 6150, Western Australia [2] Centre for Biosecurity and One Health, Harry Butler Institute, Murdoch University, Western Australia
[3] School of Medical, Molecular and Forensic Sciences, Murdoch University,

Sheep production is a multibillion-dollar industry for Australia, supplying both meat and wool. Reproductive biotechnology including sexing semen and embryo transfers play a vital role in improving production. Gamete cell DNA, such as sperm and eggs, become a key focus in sheep reproductive research. Targeting the ram gamete cell, semen provides both billions of sperm cells and contains other cells from the reproductive tissues. Moreover, during the semen collection, they can be contaminated with epithelial cells or leukocytes. This experiment aims to assess which cell lysis method yields the highest DNA copy number while minimising non-sperm cell contamination. Five cell lysis methods were designed to evaluate the copy number of DNA compared to the DNeasy Blood & Tissue kit (control), which is a standard method to extract the DNA from somatic cells. gDNA from each extraction method was quantified by Quantitative Polymerase Chain Reaction (qPCR) using the 18S rRNA gene. The result showed that the method treated with DTT yielded a significantly higher DNA copy number compared to the method without DTT ($P < 0.05$), as DTT facilitates the lysis of the acrosome to release spermatozoa DNA. The semen digest plus DTT method yielded the highest DNA copy number ($35,710,031.12 \pm 7,183,414.16$). These findings indicated that the standard DNA commercial kit (DNeasy Blood & Tissue) can extract spermatozoa DNA by combining with DTT. This optimization could lead to the development of a simple and rapid method for DNA extraction suitable for on-farm usage, offering farmers an accessible approach for applications such as sex selection in the sheep industry.

Bacterial Superheroes in the Fight against Plastic Pollution?! PET-Degradation and Upcycling to PHB with the fast-growing, marine Bacterium *Vibrio natriegens*

Anna Faber[1,2,3], Roland Politan[1], Angus Nicol,[1] George Bouras[4], Pablo Pérez-García[5], Wolfgang Streit[5], Thomas Crutchett,[2] Georg Fritz[1,2]

[1] School of Molecular Sciences, UWA, Australia, [2]UWA Oceans Institute, UWA, Australia, [3]Forrest Research Foundation, Australia, [4]University of Adelaide, Australia, [5]University of Hamburg, Germany

Plastic pollution poses a growing environmental threat, also to marine ecosystems in Western Australia (WA). With increasing levels of pollution, some microbes have started to repurpose enzymes to degrade and metabolise certain types of plastics. Yet, our understanding of how WA's unique marine biodiversity has responded to plastic pollution – and its potential to inspire biotechnological solutions – remains limited. Inspired by the Nobel Prize in Chemistry 2024, we hypothesized that we can use artificial intelligence to identify plastic-degrading enzymes from local environments and understand if and how marine microbes degrade polyethylene terephthalate (PET) plastic in Western Australian sediment. For enzyme production, we leverage the marine, non-pathogenic bacterium *Vibrio natriegens*, described to be the fastest-growing organism known to date. Moreover, we postulated that we can work towards blue, biotechnological solutions to plastic recycling and the conversion of degraded plastics into biodegradable alternatives with *V. natriegens*. Firstly, we mined PET-degrading enzyme candidates from Swan River Estuary sediments, comparing identification pipelines using AI-powered protein language models (PLMs) versus sequence-based models (HMMs). We could identify 30 promising enzyme candidates which we currently test for their PET-degradation ability. Simultaneously, we engineered *V. natriegens* to convert PET degradation product into the biodegradable polymer and "bacterial fat" polyhydroxybutyrate (PHB). Adaptive laboratory evolution further enhanced PHB yield—showing a 2.5-fold increase on PET degradation product. In conclusion, we are developing *V. natriegens* as a dual-purpose platform: to both discover and functionally screen PET-degrading enzymes from WA marine environments, and to convert PET degradation products through bioconversion into value-added, biodegradable PHB. Through this integrative approach – melding synthetic biology, AI-informed enzyme discovery, and microbial ecology – we explore sustainable plastic recycling and upcycling strategies inspired by marine environments. Further, we position *V. natriegens* as a powerful chassis for blue biotechnology and sustainable, ocean-based solutions.

Investigating Antisense Oligonucleotide (ASO) Transport in Knee Compartments

Kavishadhi Nirasha Chandrasekaran[1,2], Xander Chen[1,2], Killugudi Swaminatha Iyer[2], Haibo Jiang[2,3], Kai Chen[1,2]

[1] School of Biomedical Sciences,

[2] School of Molecular Sciences, The University of Western Australia,

Knee osteoarthritis (OA) is a degenerative joint disease with no cure, characterized by cartilage degradation, impaired bone remodelling, and synovial inflammation. Antisense oligonucleotides (ASOs) specifically target and modulate gene expression to precisely halt disease progression, offering strong potential for OA treatment. However, ASO delivery within the complex knee structure is unknown. Here, we have developed a multimodal imaging platform which enabled the spatial mapping of ASOs across knee compartments in mice. Cyanine-3-labelled ASOs (Cy3-ASO) were intra-articularly injected to assess tissue-level distribution via confocal microscopy, while iodine-127-labelled ASOs (^{127}I-ASO) were used for single-cell-level localization with nanoscale secondary ion mass spectrometry (NanoSIMS). After 24 h, mice knee joints were harvested and prepared for imaging analysis. Cy3-ASO signals were highest in the synovium, followed by articular cartilage, meniscus, bone marrow, and bone. In cartilage, localization was limited to the superficial zone, decreasing with depth. The meniscus showed higher ASO presence in vascularized outer regions compared to avascular inner regions. Minimal localization occurred in bone and bone marrow. In consistency, NanoSIMS revealed a higher uptake of ^{127}I-ASO in synoviocytes than chondrocytes and all other skeletal cells, with predominant cytoplasmic over nuclear localization. These findings together highlight the synovium could serve as a key target for ASO-based OA therapy.

Optimisation of *in vitro* transcribed CRISPR-Cas13d mRNA for liver cancer therapy

Saskia Saville[1], Nina Tirnitz-Parker[1], Rodrigo Carlessi[1]

[1]Curtin Medical Research Institute (Curtin MRI), Curtin Medical School

Hepatocellular carcinoma (HCC) is a type of liver cancer responsible for the third leading cause of cancer-related deaths world-wide. Current treatment options for advanced HCC are only effective in approximately 25% of patients and are often accompanied by adverse side effects. Due to the heterogenous nature of HCC and high proportion of un-treatable genetic disease drivers, novel therapeutic strategies with the ability to overcome these limitations are urgently required. CRISPR-Cas13d is a ribonuclease enzyme with the ability to specifically degrade RNA molecules targeted by complementary guide RNAs. Delivering it as an RNA-based therapeutic to HCC cells offers a promising strategy to selectively block the production of oncogenic proteins in hepatocellular carcinoma. Additionally, the liver is particularly primed for the uptake of RNA molecules delivered in lipid nanoparticles, with several siRNA therapeutics FDA/TGA approved for clinical application. RNA-based delivery of a CRISPR-Cas13d system may overcome safety and ethical concerns associated with viral delivery methods and could facilitate the development of multiplexed, patient-tailored therapies. Our research has focused on optimising Cas13d mRNA design, aiming for high translational efficiency and stability. Using a green fluorescent protein reporter system, we have tested different mRNA cap analogues, tailing methods, and a modified uridine base to improve Cas13d expression. We determined that CleanCap was the most efficient cap analogue, combined with encoded poly-A tailing and the addition of a modified pseudouridine base. By optimising the design of Cas13d mRNA, we hope to maximise the therapeutic potential of this enzyme to knockdown chosen cancer associated genes. This could ultimately lead to the development of personalised treatment strategies and could improve the therapeutic landscape for liver cancer patients.

Validating antisense oligonucleotides against MIR205HG in Breast Cancer

Alicia Dixon[1,2,3], Pieter Eichhorn[1,2,3], Chris Witham[1,2,3]

[1]Curtin Medical Research Institute, [2]Curtin University, [3]Curtin Medical School

Non-coding DNA was first deemed as 'junk' and thought to play no role in the body. More recently, non-coding RNAs were characterised, and the role of microRNAs, long non-coding RNAs, short interfering RNA and more, were uncovered. A multitude of long non-coding RNA (lncRNA) and micro-RNA (miRNAs) molecules have been found to possibly play a role in the development and drug resistance of various cancers, including breast cancer. Breast cancer is the most prevalent cancer in women worldwide, and due to the inherently dynamic nature of breast tissue, and the complexity of cancer, the efficacy of treatments is impeded, and drug resistance is fostered. PI3K inhibitors are a drug class designed to inhibit the PI3-Kinase pathway, which is often hyperactivated in breast cancer. Preliminary data observed that PI3K inhibitor treatment in MCF7 breast cancer cell lines induce upregulation of the long non-coding RNA, MIR205HG. Furthermore, when MCF7 cells with MIR205HG knocked out were treated with a PI3K inhibitor, cellular synthetic lethality was induced 5-days after treatment. Various genes have been observed to be dysregulated alongside MIR205HG knockout and PI3K inhibitor treatment, with a large portion of them playing a role in cellular proliferation and epithelial to mesenchymal transition (EMT). This project aims to characterise the role of MIR205HG in breast cancer, and the interplay of the PI3K pathway, whilst investigating the therapeutic potential of antisense oligonucleotides (ASOs) targeting MIR205HG. Through quantitative real-time PCR (qRT-PCR) we can observe the differential expression of our genes of interest, including AREG, EREG, PCDH10, EPGN and BTC. The therapeutic potential of the ASOs will be evaluated using proliferative assays such as boyden chamber assay and scratch healing assays, in combination with qRT-PCR. This project aims to uncover the elaborate nature MIR205HG, and contribute to the understanding of the relationship between lncRNAs and cancer.

Optimisation of *in vitro* transcribed CRISPR-Cas13d mRNA for liver cancer therapy

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C3

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C4

Designing Synthetic PPR-RNA Tools To Monitor and Regulate Biological Events Using Conformational Changes

Sanathanee Sachchithananthan [1], Kim T Melville[1], Ian D Small K[1, 2], Andrew C Marshall[1], Charles S Bond[1]

School Molecular Sciences & [2]ARC Centre of Excellence for Plant Energy Biology, University of Western Australia.

Introduction: Proteins undergo conformational changes upon interacting with other proteins, nucleotides, or other molecules. Understanding these structural shifts is key to elucidating protein function and designing controllable molecular tools. Pentatricopeptide repeat proteins (PPR) are composed tandem α-helical motifs and exhibit sequence-specific RNA binding. These proteins undergo conformational change upon binding to RNA targets, adopting a compressed super helical conformation compared to their unbound state. **Problem Statement**: This study investigates how RNA-induced conformational changes in PPR proteins contribute to protein function, and how such changes can be exploited to design RNA-responsive PPR-based synthetic tools. **Procedures**: Previous studies with chemically labelled PPR proteins demonstrated conformational upon RNA-binding change using fluorescence resonance energy transfer (FRET). Building on this, a 9 repeat S class PPR protein was protein was engineered with Now-GFP and mRuby2 (9S-FP) fused to the N- and C-terminus. To investigate the binding affinity and conformational shifts, 9S-FP was analysed using analytical size-exclusion chromatography (SEC) and FRET, whereas micro scale thermophoresis was used to analyse the binding affinity of 9S control PPR protein without FP. **Results**: Analytical SEC confirmed RNA binding of 9S-FP, with the protein:RNA complex eluting at a distinct retention time from protein- or RNA-only samples. In vitro FRET assays showed an increasing FRET signal upon RNA titration, consistent with a binding-induced conformational change. MST analysis of the control 9S protein revealed an RNA-binding affinity of 60 nm. **Conclusions**: The results indicate that the PPR protein retains RNA-binding ability and undergoes conformational changes despite FP attachments at both terminals. However, a stronger FRET pair is needed to improve the signal. Furthermore, a similar system can be employed to longer PPR proteins where the FPs will be fused in between the PPR tracts for FRET studies. This system has potential as the basis for RNA-responsive biosensors.

Optimizing mRNA Vaccine Design Using a Multi-Fluorophore System: Neo-Rainbow

Marie Jyotsna Jawahar[1], Archa Fox[1], Tessa Swain[1], Francois Rwandamuriye[1], Alec Redwood[1], Jenette Creaney[1]
The University of Western Australia[1]

mRNA vaccines have emerged as a promising approach in the field of cancer immunotherapy. In the case of lung cancer, these vaccines aim to deliver tumour-specific antigens (neoepitopes) within the host cells where they are processed and presented by the major histocompatibility complexes as antigens to trigger patient-specific, targeted immune responses. Several clinical trials, including those by Moderna and BioNTech, are ongoing and paving the way for mRNA vaccines as an effective form of immunotherapy. However, the efficacy of these mRNA vaccines is dependent on the design of the mRNA molecule, especially in the case of multi-antigen or neoepitope arrays used for treating lung cancer tumours. The expression of these arrays is influenced by several design features, such as the order and position of the neoepitopes in the array and the type of linker sequence used. This study will investigate how these design parameters affect translation efficiency and protein expression using a multi-fluorophore-based system, here termed the Neo-rainbow. In this model, four distinct fluorophores will replace the antigen sequences, enabling visual assessment of the expression patterns in mammalian cells. By systematically varying the fluorophore arrangement and the linker type, we aim to identify how these design features effect protein translation, which can then be used to help improve the design of future neoepitope arrays. Thereby, identifying strategies to improve mRNA design and providing valuable insights into more effective mRNA vaccines for personalized cancer therapies.

This research was supported by the Australian Centre for RNA Therapeutics in Cancer (ACRTC) and the RNA Innovation Foundry (RIF). The company had no role in analysing the data or preparing the abstract.

Optimising dsRNA-based immunoadjuvants

Cenxi Gao[1,2], Xueting Ye[1,2], Jessica Kretzmann[2], Christian Tjiam[1], Ben Wylie[1,2], Rakesh Veedu[3,4], Charlie Bond[2], Joost Lesterhuis[1,2], Tao Wang[1,2]

Affiliations: [1]The Kids Institute Australia, [2]The University of Western Australia, [3]Centre for Molecular Medicine and Innovative Therapeutics & [4]Institute for Immunology and Infectious Diseases, Murdoch University

Double-stranded RNA (dsRNA) is a hallmark molecule of viral infection. During viral invasion, dsRNA activates an innate immune response by engaging pattern recognition receptors including Toll-like receptor 3 (TLR3), melanoma differentiation-associated protein 5 (MDA5), and retinoic acid-inducible gene I (RIG-I). This activation triggers the secretion of pro-inflammatory cytokines and type I interferons. These factors play crucial roles in enhancing antigen presentation and co-stimulatory signals, thereby promoting a robust adaptive immune response. As a result, dsRNA offers promising applications in both cancer immunotherapy and vaccine development. The dsRNA used in clinical trials is dominated by polyinosinic:polycytidylic acid, Poly(I:C). However, this classical dsRNA product is produced in a manner resulting in a mixture of different lengths of dsRNAs, which causes significant batch-to-batch variation, compromising its clinical translation. This project aims to develop an optimal dsRNA preparation and test its anti-cancer efficacy and vaccine adjuvanticity using our established animal models. Strategies under investigation include developing novel dsRNAs of defined structures, optimal delivery system and chemical modification for enhanced stability, adjuvanticity and safety in both cancer immunotherapy and vaccine settings. Mechanistic studies will elucidate how these modifications affect dsRNA recognition by innate receptors, downstream signalling pathways, and immune cell activation in vitro using knock-out cell lines for TLR3, RIG-I and MDA-5, and in vivo in mouse cancer and vaccine models. Our preliminary data demonstrate that our in-house synthesised fixed-structure dsRNA elicits significantly higher antigen-specific IgG titers compared to Poly(I:C) and FDA-approved vaccine adjuvants, highlighting its potential to enhance vaccine immunogenicity as an adjuvant. In vivo cancer experiments are ongoing. Ultimately, this work seeks to enhance vaccine efficacy against challenging pathogens and curtail tumour progression through the integration of dsRNA-based immunoadjuvants with optimised delivery systems and cutting-edge nucleic.

Investigating Antisense Oligonucleotide (ASO) Transport in Knee Compartments

Kavishadhi Nirasha Chandrasekaran[1,2], Xander Chen[1,2], Killugudi Swaminatha Iyer[2], Haibo Jiang[2,3], Kai Chen[1,2]

[1] School of Biomedical Sciences,

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Knee osteoarthritis (OA) is a degenerative joint disease with no cure, characterized by cartilage degradation, impaired bone remodelling, and synovial inflammation. Antisense oligonucleotides (ASOs) specifically target and modulate gene expression to precisely halt disease progression, offering strong potential for OA treatment. However, ASO delivery within the complex knee structure is unknown. Here, we have developed a multimodal imaging platform which enabled the spatial mapping of ASOs across knee compartments in mice. Cyanine-3-labelled ASOs (Cy3-ASO) were intra-articularly injected to assess tissue-level distribution via confocal microscopy, while iodine-127-labelled ASOs (^{127}I-ASO) were used for single-cell-level localization with nanoscale secondary ion mass spectrometry (NanoSIMS). After 24 h, mice knee joints were harvested and prepared for imaging analysis. Cy3-ASO signals were highest in the synovium, followed by articular cartilage, meniscus, bone marrow, and bone. In cartilage, localization was limited to the superficial zone, decreasing with depth. The meniscus showed higher ASO presence in vascularized outer regions compared to avascular inner regions. Minimal localization occurred in bone and bone marrow. In consistency, NanoSIMS revealed a higher uptake of ^{127}I-ASO in synoviocytes than chondrocytes and all other skeletal cells, with predominant cytoplasmic over nuclear localization. These findings together highlight the synovium could serve as a key target for ASO-based OA therapy.

Does cellular plasticity drive invasion in mesothelioma?

Callum Flanagan[1,2], Alistair Nash[1,2], Ebony Rouse[1,3], Alec Redwood[1,3], Bruce Robinson[1,2,3,4], Jenette Creaney[1,2,3,4]

[1]National Centre for Asbestos Related Diseases, University of Western Australia, [2]Medical School, University of Western Australia, [3]Institute for Respiratory Health, [4]Department of Respiratory Medicine, Sir Charles Gairdner Hospital.

Introduction. Pleural mesothelioma is an aggressive cancer arising from mesothelial cells lining the pleural cavity with poor survival and limited treatment options. Mesotheliomas are classified into three major histological subtypes: epithelioid, sarcomatoid and biphasic. Epithelial to mesenchymal transition (EMT) is a form of cellular plasticity which drives invasion and metastasis in many cancers and non-epithelioid mesothelioma cells often display EMT-like characteristics. Loss of BRCA1-associated protein-1 (BAP1) function is a frequent event in mesothelioma, more prevalent in epithelioid tumours and is correlated with improved patient outcomes. **Problem Statement**. Mesothelioma is a highly invasive cancer, but the driving mechanisms of invasion are poorly understood. It was hypothesised that EMT promoted invasive phenotypes in mesothelioma, BAP1 modulated cell phenotypes and targeting mechanisms of cellular plasticity would inhibit invasion. **Procedures**. To test the hypothesis that mesothelioma cells with a more mesenchymal phenotype are more invasive, EMT was induced using transforming growth factor beta-1. Six mesothelioma cell lines were assessed. Two cell lines had BAP1 deletion induced using CRISPR. The invasiveness of cell lines was assessed using scratch wound healing, transwell invasion and 3D spheroid invasion assays. Changes to cell phenotypes were assessed using RNA sequencing and immunoblotting for EMT-associated markers. **Results**. Inducing EMT significantly increased wound closure in 2/6 cell lines and increased transwell invasion at least 2-fold in 4/6 cell lines. Inducing EMT increased 3D spheroid invasion by 24% in the only cell line tested to date. CRISPR mediated BAP1 deletion induced a more epithelial-like phenotype in both cell lines, while significantly decreasing wound closure, transwell invasion and 3D spheroid invasion. A TGFBR1 inhibitor was found to cause cells to adopt an epithelial-like phenotype and inhibited migration at non-cytotoxic doses. **Conclusions**. EMT and BAP1 were shown to promote invasive phenotypes in mesothelioma and a strong candidate for targeted therapy was identified.

Developing a Three-Dimensional Organoid Model of Skin for High-Throughput Drug Testing

Peter K.H. Lee[1], Lara Perryman[2], Andrew W. Stevenson[3], Yu Suk Choi[4], Fiona M. Wood[3,5], Wolfgang Jarolimek[2], Mark W. Fear[3], Killigudi Swaminathan Iyer[1]

[1]School of Molecular Sciences, [2]Drug Discovery, Syntara Ltd., [3]Burn Injury Research Unit, School of Biomedical Sciences, & [4]School of Human Sciences, University of Western Australia, [5]Fiona Wood Foundation, Murdoch

Developing novel therapeutics for skin disorders remains bottlenecked between pre-clinical and clinical stages due to the lack of physiological relevance of commonly used models for skin and/or the long timeframes and complexity of more relevant *in vitro* skin models. Cell monolayers often present an altered representation of cell phenotype, while animal studies are expensive, slow and can lack biological significance due to interspecies differences. Three-dimensional (3D) culture systems can potentially be designed to recapitulate the 3D biophysical and biochemical cues to provide more physiologically relevant alternative models, as well as integrate into a scalable methodology for high-throughput screening. However, these systems must be characterised fully for their successful application. Commercially available full-thickness skin equivalents (FTSE) mimic the 3D organisation of the dermal and epidermal layers and are frequently used for screening purposes. However, the long maturation period for skin construct fabrication (~2-3 weeks) limits data throughput for studying skin biology. Therefore, there is a need to develop a scalable platform for FTSEs that is rapid and easy to replicate. Here, the development and characterisation of a layered coculture organoid of human dermal fibroblasts (nF) and keratinocytes (nK) from healthy donor skin is explored. Sequential self-assembly of nF followed by nK in low-adhesion 96-well plates achieved skin organoids within six days. Immunofluorescent staining showed spatially dependent expression of proteins related to keratinocyte differentiation (cytokeratin-10) and the dermal epidermal junction (collagen-iv, collagen-vii), found towards the organoid periphery and the nF-nK interface respectively. Additionally, RT-qPCR showed elevated mRNA of nK differentiation genes in organoids compared to monolayers (*KRT10*, *CASP14, IVL*), suggesting a stratified epithelial layer was generated. These results demonstrate that skin organoids capture 3D aspects of native skin, and due to its self-assembling nature integration into automated systems (such as bioprinting) can be further explored, potentially enabling high-throughput screening of skin biology.

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D3

Exploring the plasticity of Spiny mouse dermal fibroblasts, as a blueprint for human skin regeneration

E. Heather Russell[1,2], Runshi Zheng[3], Andrew Stevenson[1,2], John Henderson[3], Zahra Massoud[3], Ahmed Muhammad[1,2], Fiona Wood[1,2], Sofia Ferreira-Gonzalez[1,2] and Mark Fear [1,2]

[1]University of Western Australia, [2] Fiona Wood Foundation, [3]University of Edinburgh

Fibrosis, which results from the excessive buildup of scar tissue throughout the body is estimated to contribute to over 17% of all deaths in the developed world and have a huge financial impact on healthcare systems worldwide, due to the resource intensive and continuous nature of the treatment. Not only do scars have a severe physiological deficit with the skin never regaining full function but are also known to cause significant psychosocial damage. Emerging evidence in human dermal fibroblasts suggests that there are distinct functional subpopulations of fibroblasts with discrete roles in orchestrating skin during wound repair, with specific subtypes being more closely involved in scarring or regeneration. How these subpopulations arise and how committed cells are to their functional identity remains unknown. Using single cell rnaSEQ and flow cytometry, we have found that human dermal fibroblast exhibit plasticity across the fibroblast lineage and are not committed to a fibrotic identity. For this reason, we turn to the spiny mouse, know to regenerate its skin and internal organs after injury. The spiny mouse poses unprecedented potential for application to meliorate human scarring and fibrosis to improve patient outcomes, providing clues for regenerative cues. Consequently, we investigated the heterogeneity of spiny mouse dermal fibroblasts to elucidate potentially regenerative subpopulations and act as a blueprint to drive human dermal fibroblast towards a more regenerative phenotype and found they exhibit intrinsic plasticity across several cell lineages- spontaneously differentiating into neurons, epithelial cell and many more. Additionally, the conditioned media removed from spiny fibroblast during culture seems to have to ability to change human cell behaviour, such as migration and may be a potential clue into regenerative drivers. Understanding pathways by which the spiny mouse fibroblasts support regeneration and the pathways that orchestrate this will help to drive skin from wound repair to regeneration.

D4

Decoding Fibroblast Plasticity in Skin Regeneration and Fibrosis

Ahmed Muhammad Iluoreh[1,2,3], Heather Russell[2], Fiona Wood[2,3], K. Swaminathan Iyer[1,3], Wolfgang Jarolimek[4], Nicole M. Smith[1,3], Andrew Stevenson[2,3], Mark W. Fear[2,3].

[1]School of Molecular Sciences,[2]Burn Injury Research Unit, School of Biomedical Sciences, [3]ARC Training Centre for Next-Gen Technologies in Biomedical Analysis, The University of Western Australia, [4]Drug Discovery Department, Syntara Ltd, Sydney, Australia.

This research investigates human dermal fibroblast heterogeneity by examining $CD90^{+/-}$ population dynamics over time, comparing sorted versus unsorted fibroblast cultures. We hypothesize that fibroblast subpopulations represent dynamic states rather than fixed lineages, and that DNA secondary structures (G-quadruplexes and i-Motifs) may serve as molecular switches regulating these transitions. We employed Fluorescence-Activated Cell Sorting (FACS) to isolate $CD90^{+}$ and $CD90^{-}$ fibroblast subpopulations based on CD90 expression; a surface marker linked to fibrotic activity. Initial analysis identified $CD90^{+}$ (~90%) and $CD90^{-}$ (~10%) populations. To assess population stability and functional dynamics, we cultured and monitored three experimental conditions: Sorted $CD90^{+}$ (pure $CD90^{+}$ cells), sorted $CD90^{-}$ (pure $CD90^{-}$ cells), and unsorted populations (original mixed control population). CD90 expression and collagen (COL1) deposition were assessed at multiple time points using dual immunocytochemistry. Time-course analysis revealed dynamic population reversion in sorted cultures. Both isolated $CD90^{+}$ and $CD90^{-}$ populations progressively shifted towards the original $CD90^{+}/CD90^{-}$ ratio over time, indicating these states are not permanently fixed. In contrast, unsorted populations maintained relatively stable ratios over time. Functional analysis using dual staining for CD90 and collagen revealed distinct temporal patterns. $CD90^{+}$ cells consistently deposited more collagen compared to $CD90^{-}$ cells. However, the relationship between CD90 expression and collagen production showed dynamic changes over time in sorted populations undergoing phenotypic reversion. These findings suggest that $CD90^{+/-}$ states represent plastic, interconvertible phenotypes rather than stable lineages. The reversion toward equilibrium in sorted populations indicates intrinsic regulatory mechanisms that maintain population balance. The dynamic reversion of $CD90^{+/-}$ populations demonstrates the inherent plasticity of fibroblast subpopulations. This plasticity combined with functional outputs (collagen deposition), suggests that targeting the molecular mechanisms controlling these state transitions, potentially through DNA secondary structures, may offer novel therapeutic approaches for modulating fibrotic responses. Understanding these population dynamics provides crucial insights for developing more effective anti-fibrotic strategies.

CRISPR Single-Base Editing and Neural Disease Modelling for Functional Interpretation of RASopathy Variants of Uncertain Significance

Paula Gomez[1,2], Catherine A. Forbes[1], O. Kayden Wright[1,2], Maxine E. Smith[1], Kathryn M. Morris[1], Clayton T. Fragall[2], Nicole C. Shaw[1,2*], Linda K. Wijaya[1,2*], and Vanessa S. Fear[1,2*].

[1]The Kids Research Institute of Australia, , [2]The University of Western Australia, [*]Equal contributors

Introduction. The RASopathies are rare genetic syndromes caused by germline variants in genes of the RAS/MAPK signalling pathway. These disorders are clinically heterogeneous and frequently present with neurodevelopmental features, making genetic diagnosis essential. Despite advancements in next-generation sequencing (NGS), up to 50% of RASopathy patients remain undiagnosed. This is largely due to the detection of variants of uncertain significance (VUS), which require functional validation to determine pathogenicity. Currently, patients wait 5-30 years for VUS interpretation, if this can be achieved at all. In this study, we are investigating three paediatric patients with suspected RASopathy disease. Genetic sequencing identified that the patients were heterozygous for the genetic VUS, *LZTR1* c.317G>A, p.Cys106Tyr; *RIT1* c.116T>G, p.Met39Arg; and *PTPN11* c.1374C>A, p.His458Gln. **Problem Statement**. The study aims to fast-track the functional interpretation of RASopathy-associated VUS. Patient-specific neural disease models will be developed to identify disruptions in RAS/MAPK signalling and elucidate molecular and cellular changes contributing to disease. **Procedures**. CRISPR-Cas9 homology-directed repair was utilised for high efficiency single-base gene editing of iPSCs, followed by single-cell cloning and amplicon sequencing to identify heterozygous clones. In parallel, protocols were established to form iPSC-derived mature neurons, and optimise RAS/MAPK signalling assays. **Results**. Preliminary results confirm successful iPSC model generation for the *RIT1* VUS. We anticipate that optimised assay conditions will enable detection of subtle differences in ERK1/2 phosphorylation between VUS and matched wildtype clonal cell lines, providing functional evidence to support patient diagnosis. **Conclusions**. Our findings will lay the foundation for a scalable, patient-specific platform to functionally characterise RASopathy-associated VUS within neural disease models. This work will offer an unprecedented opportunity to shorten the diagnostic odyssey for patients with rare disease, to elucidate the molecular mechanisms driving disease, and identify disease-specific treatment strategies.

Unravelling CACNA1A Channelopathies: Stem Cell Models for Rapid Functional Analysis of Rare Genetic Variants

O. Kayden Wright[1,2], Catherine Forbes[1], Paula Gomez[1,2], Maxine Smith[1], Linda K Wijaya[1], Nicole Shaw[1], Vanessa S Fear[1]

[1]Translational Genetics Team, The Kids Research Institute Australia, [2]School of Biomedical Sciences, University of Western Australia

Introduction: Rare genetic diseases affect up to 8% of the world population. Clinical use of genetic sequencing has enabled rapid diagnosis for up to half of patients by identification of disease-causing pathogenic variants. In most of the remaining patients a variant of uncertain significance (VUS) is identified. These variants require functional validation in highly specialized laboratories to establish pathogenicity; a process that may take many years. CRISPR-Cas9 gene editing and stem cell disease modelling provides a fast, informative, disease agnostic pathway for the functional validation of VUS. This study investigated two *CACNA1A* VUS (*CACNA1A* p.Ala1959Asp, and *CACNA1A* p.Lys1438del) from patients with suspected *CACNA1A* channelopathies. **Methods:** Patient *CACNA1A* VUS were introduced into induced pluripotent stem cells (iPSCs) through CRISPR-Cas9 gene editing, and clonal VUS and isogenic WT cell lines derived. Targeted amplicon sequencing was used to determine gene editing efficiency, to identify genetic variant single cell clones, and to confirm DNA integrity at off-target CRISPR-Cas9 sites. Paired VUS and WT cell lines were differentiated to neural progenitor cells (NPCs), and then mature neurons. Differentiation was assessed by flow cytometry for expression of stem markers (OCT3/4 and NANOG), and neural markers (PAX6 and Nestin). Expression of neural progenitor marker PAX6, immature neuron marker DCX, mature neuron marker TUBB3, and calcium channel $Ca_v2.1$ was investigated via immunofluorescence. **Results:** Matched isogenic *CACNA1A* VUS and WT iPSC lines were derived for each genetic variant. Decreased expression of stem markers and increased expression of neural markers indicated VUS and WT cells were differentiated to NPCs. Immunofluorescence examined expression of PAX6, DCX, TUBB3, and $Ca_v2.1$ in VUS and WT NPCs and mature neurons. **Conclusions:** This work establishes neural stem cell models of patient *CACNA1A* VUS compared to isogenic controls. Future work will establish the functional impact of each patient VUS through patch-clamp electrophysiology and transcriptomic analysis.

Characterisation of Bone Marrow Extracellular Matrix and Fibroblast Populations During the Development of B-cell Acute Lymphoblastic Leukaemia

Yasmin Henderson-Kelly[1,2], Vincent Kuek[1,2,3], Rishi S. Kotehca[1,2,3], Laurence C. Cheung[1,2]

[1]The Kids Research Institute, [2]Curtin Medical School and Curtin Medical Research Institute, Curtin University, [3]School of Biomedical Sciences and UWA Medical School, University of Western Australia

Introduction. B-cell acute lymphoblastic leukaemia (B-ALL) is one of the most diagnosed and deadliest childhood cancers in Australia. Despite recent advances in modern medicine leading to improved overall survival, leukaemia patients with high-risk genetic subtypes have poor prognosis and survival outcomes. Therefore, it is fundamental to understand the disease mechanisms and design new treatment strategies to tackle these high-risk leukaemias. **Problem Statement**. In studies of solid cancers, changes in fibroblasts and extracellular matrix (ECM) have been consistently linked to increased metastasis, immunosuppression, and evasion of therapeutics. However, their roles in leukaemia progression remain unclear. This study aimed to characterise the changes in bone marrow fibroblast populations and ECM depositions during the progression of B-ALL. **Procedures**. An immunocompetent mouse model carrying BCR-ABL1$^+$ B-ALL was utilised to represent high-risk leukaemia. Using flow cytometry, fibroblast populations were analysed and phenotypic changes in surface marker expressions (e.g., α-SMA and FAP) were characterised in healthy, low burden, mid burden, and high burden groups. Patterns in distribution and accumulation of three ECM proteins, namely perlecan, fibronectin, and collagen III were analysed by immunofluorescence staining of cryo-preserved bone sections. **Results**. Bone marrow fibroblasts underwent significant transition from predominantly α-SMA$^+$ population in the healthy and low disease burden groups to an FAP$^+$ population, peaking in mid burden but maintained in high burden. Analysis of the ECM remains ongoing. **Conclusions**. Our study has shown that changes in the bone marrow fibroblasts are associated with leukaemia progression. This will be further validated as the ECM they produce may support a malignant environment. Further studies should be undertaken to investigate how and why these shifts are initiated.

Scaling eDNA for Marine Biodiversity Monitoring: Five Years Across Australia's Oceans

Marcelle E. Ayad[1,2], Philipp E. Bayer[1,2], Adam Bennet[2], Shannon Corrigan[1,2], Eric J. Raes[1,2], Matthew Fraser[1,2], Priscila Goncalves[1,2], Georgia Nester[2,3], & OceanOmics Centre[2], Michael Bunce[1,2]

1. Minderoo Foundation, Broadway, 2. Minderoo OceanOmics Centre at UWA, Oceans Institute, The University of Western Australia, 3. Minderoo-UWA Deep-Sea Research Centre, School of Biological Sciences and Oceans Institute, University of Western Australia

Environmental DNA (eDNA) technology is rapidly gaining global traction for its potential to transform how we monitor and understand marine biodiversity at scale. Its use is expanding through integration with long-term monitoring programs, deployment of automated samplers aboard research vessels, and growing investment in computational and AI-driven tools for managing complex and large datasets. At the same time, advances in sampling technologies and visualization tools are making eDNA more accessible for use in community-led initiatives and citizen science efforts focused on coastal and nearshore environments. Together, these developments are scaling eDNA applications across a range of users and ecosystems, broadening both the scope and resolution of biodiversity insights. We applied a multi-marker eDNA approach to establish marine vertebrate biodiversity baselines and explore spatial and temporal patterns across Australia's EEZ—from the Christmas Island to Hobart and to the east coast of NSW. Between 2019 and 2024, over 6,000 samples were collected from coastal shelves, continental slopes, offshore waters, and deep-sea regions, generating a high-resolution dataset that reveals clear patterns in community composition across space, time, and depth. Fine-scale variation was evident around remote islands, highlighting the utility of eDNA in local-scale biomonitoring. Along large ocean transects, surface samples captured taxa from pelagic, mesophotic, and deep-water habitats, capturing longitudinal shifts in communities and suggesting vertical transport of genetic material. While surface samples detected some mesophotic- and deep-water species, depth-stratified sampling revealed strong vertical structuring, with distinct vertebrate assemblages across depths. These findings demonstrate the value of eDNA metabarcoding for detecting biodiversity patterns across multiple scales and supporting sustained ecosystem monitoring. As marine ecosystems continue to shift under a changing climate, eDNA datasets will offer critical contributions to global biodiversity repositories and enhance our ability to detect, interpret, and respond to ecological change across scales.

Input reference strategies for homology-based genome annotation in bony fish (Class Actinopterygii)

Anna Depiazzi[1,2], Rich Edwards[1], Dave Edwards[2], OceanOmics Centre[1], Emma de Jong[1]

[1]Minderoo OceanOmics Centre at UWA, Oceans Institute, University of Western Australia, Crawley, WA, Australia, [2]School of Biological Sciences, University of Western Australia, Crawley, WA, Australia

Researchers widely use genomes and their annotations to study the ecology and evolution of organisms. One common strategy for predicting genes in newly sequenced species involves using homology-based annotation, where researchers leverage information from existing genome annotations. However, the quality of the resulting annotation depends on the choice and number of reference genomes used, which impacts downstream use. Nonetheless, best practices for reference annotation selection remain unestablished, particularly in conservation genomics relating to non-model organisms. In this study, the performance of the commonly used homology-based annotation tool Gene Model Mapper (GeMoMa) was evaluated in publicly available bony fish (Class Actinopterygii) genomes by comparing two commonly used reference selection strategies and varying numbers of input reference species (1-10). Measures of sensitivity, precision, F1 scores (a harmonic mean accounting for imbalances), and protein richness were used to determine the optimal approach. Results demonstrated that five phylogenetically diverse references produced the best results regarding information richness ($p < 0.05$), balancing computational requirements with accuracy. Ten references produced annotations with higher total domain counts ($p = 0.031$ and 0.035), though the diversity was not any greater, and F1 scores also declined. Annotations were then evaluated with the CAFE5 software package to ascertain the impact of annotation quality on gene family evolution. The annotations created using five references produced significantly higher-resolution outcomes than those created using one reference ($p < 0.005$). Importantly, the snake pipefish superseded annotations relying solely on the charismatic zebrafish. These findings highlight an optimal strategy for producing biologically informative annotations critical for applications in conservation biology.

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Thinking Inside the Box - Enhancing Arboreal Marsupial Monitoring in a Post-Mining Landscape Using Environmental DNA

Austin Guthrie[1,2], Paul Nevill[1,2], Bill Bateman[2], Christine Cooper[2], Mieke van der Heyde[1,2]

[1]Trace & Environmental DNA (TrEnD), Curtin University [2]School of Molecular and Life Sciences, Curtin University

Restoring faunal communities is a key goal in post-mining rehabilitation, yet monitoring cryptic species such as arboreal marsupials remains challenging. Artificial habitat structures such as nest boxes have been widely used to support hollow-dependent fauna in regenerating landscapes, particularly where natural tree hollows are scarce. This study examined nest box occupancy on the South32 Worsley Alumina bauxite mine, located in the Northern Jarrah Forest bioregion of south-western Australia, using a multi-method approach. Environmental DNA (eDNA) metabarcoding was used to detect the presence of four arboreal marsupials—the red-tailed phascogale, brush-tailed phascogale, yellow-footed antechinus, and the western pygmy possum—from swab samples collected inside 90 nest boxes. Sampling was conducted every six months over a two-year period across a rehabilitation gradient (1980s–2010s) and reference forest. eDNA results were compared with conventional monitoring techniques, including motion-activated camera traps and visual nest box inspections. The findings highlighted the complementary strengths of each method, with eDNA metabarcoding providing increased species detectability relative to visual and camera-based observations alone. This study demonstrated the value of integrating molecular tools into ecological monitoring frameworks for cryptic fauna. Ongoing analyses are assessing the role of vegetation structure and rehabilitation age in shaping species presence, to provide further insight into habitat quality and restoration success.

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High-throughput sequencing reveals undetected fungal pathogens and cross-antagonism in soil mycobiomes

Viet-Cuong Han[1,2], Nicole E. White[2,3], Pippa J. Michael[1], Duong Vu[4], Bec Swift[2], Sarita Jane Bennett[1,2]

[1]Centre for Crop and Disease Management, Curtin University, WA 6102, Australia, [2]School of Molecular and Life Sciences, Curtin University, WA 6102, Australia, [3]Trace and Environmental DNA Laboratory, Curtin University, WA 6102, Australia, [4]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

High-throughput sequencing (HTS) has emerged as a powerful tool for studying the soil microbiome. This study employed HTS to investigate how long-term agricultural practices, specifically brassica/legume/cereal crop rotations, influence the composition, ecological roles, and pathogenic potential of soil fungal communities. We compared agricultural soils with adjacent non-agricultural remnant vegetation soils in Kojonup, Western Australia, and found substantial differences in fungal community composition. Agricultural soils harboured significantly higher abundances of fungal plant pathogens. We detected both known and previously unrecorded fungal pathogens affecting brassica, legume, and cereal crops, as well as other host plants not cropped in the vicinity of the study area. Notably, we recovered two species within the *Fusarium incarnatum-equiseti* species complex (FIESC), including *F. clavus* (syn. *F. clavum*) and a potentially novel clade closely related to FIESC 31 and *F. wereldwijsianum*, both of which are pathogenic to cereals. We also recovered *Leptosphaeria maculans*, the causal agent of blackleg in canola (*Brassica napus* L.), from soils. This study provides the first report of FIESC members, including *F. clavus* and a potentially novel clade within the complex, as emerging cereal pathogens in Australia. Furthermore, we identified fungal species known to be pathogenic to animals and humans, recorded cross antagonism among soil fungal plant pathogens, and discovered fungal biocontrol agents inhibiting fungal pathogens. These findings reveal the accumulation of disease-causing fungi in soil ecosystems following agricultural land use, while also highlighting the complexity and intricate interactions within soil fungal communities.

Benchmarking Methods for Genome Annotation Using Nanopore Direct RNA in a Non-Model Crop Plant

Jade Davis[1], Kristina Gagalova[1], Lilian Sanglard[1], Sabrina Cuellar[1], Mark Gibberd[1], Fatima Naim[1]
[1]Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University

High-quality reference genome annotations are crucial resources for transcriptomic analysis as they provide detailed information on genomic and transcriptomic features, enabling the interpretation of RNA sequencing data. While reference annotations are relatively well-curated for the model plant Arabidopsis thaliana, they remain limited for many non-model plant species, including barley. Improving annotations is particularly challenging due to the inherent complexity of plant genomes, stemming from factors including polyploidy, large genome size, and high repeat content. Long-read direct RNA (dRNA) sequencing from Oxford Nanopore Technologies enables the capture of full-length transcripts, presenting as a powerful tool for transcript resolution and annotation improvement. While numerous bioinformatic tools support dRNA-informed annotation, studies benchmarking optimal methods for this process have focused on animal models. Previous studies identified that tool performance varied across sequencing technologies and datasets, reference quality and overall annotation strategies, highlighting a potential need for plant-specific benchmarking In this study, we benchmarked five annotation tools: StringTie2, IsoQuant, Bambu, FLAIR, and FLAMES, using dRNA data from barley infected with Net Form Net Blotch disease. Benchmarking identified substantial variation across tools in isoform detection, structural completeness, splicing classification, and handling of 5′ read truncation. Several tools successfully identified novel transcripts, with the top-performing reference-guided approach detecting 994 previously unannotated transcripts, including candidates with predicted roles in disease response. This study offers novel insights into optimal bioinformatic methods for dRNA-based genome annotation in a non-model crop plant. These findings could be applied to further improve barley annotations, capturing novel transcript populations under different stress states, or applied to other plant species. The identification of novel, disease response related transcripts also highlights the importance of continued refinement of annotations using RNA-seq from a condition of interest. Finally, these results highlight the importance of plant-specific benchmarking of bioinformatic tools.

Preclinical Evaluation of Menin Inhibitors for Infant Acute Lymphoblastic Leukaemia

Stephen Dymock[1,2], Joyce Oommen[1], Laurence Cheung[1,3], Rishi Kotecha[1,2,3]

[1] The Kids Research Institute Australia, [2]The University of Western Australia, [3]Curtin University

Over the last 70 years, the treatment of acute lymphoblastic leukaemia (ALL) has gradually evolved, with progressive improvements in the efficacy of multi-agent chemotherapy regimens and risk stratification leading to 5-year overall survival rates approaching 90%. However, infants with acute lymphoblastic leukaemia (iALL) comprise a subgroup of ALL that continue to have significantly inferior outcomes. The KM2TA rearrangement is present in up to 80% of iALL patients and this aggressive genetic driver mutation together with increased vulnerability to treatment-related toxicity has resulted in a 5-year event-free survival of only 40%. Evidently, there remains an urgent need for the identification of novel, safe and effective therapies for iALL. Menin inhibitors directly target the KMT2A complex and have recently emerged as a promising strategy for iALL treatment. We therefore sought to comprehensively evaluate the preclinical efficacy of menin inhibitors for iALL. An extensive panel of unique infant KMT2A-rearranged and wild-type iALL cell lines and mouse models were exposed to eighteen different menin inhibitors and cytotoxicity was assessed using a modified Alamar Blue assay. The Bliss Independence model was applied to identify synergistic interactions between clinically-relevant menin inhibitors (BMF-219, revumenib and ziftomenib) with conventional chemotherapeutic agents used to treat iALL. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was undertaken to determine the expression of menin-related genes in iALL cell lines. We found that menin inhibitors displayed variable *in vitro* and *ex vivo* cytotoxicity across the panel of cell lines and mouse models. In preclinical iALL models, BMF-219, revumenib and ziftomenib largely exerted additive effects when combined with conventional chemotherapeutic agents and led to variable downregulation of menin-related genes. Although menin inhibitors remain an exciting class of drugs for the treatment of KMT2A-rearranged iALL, our findings suggest that thorough *in vitro* and *in vivo* testing combined with genetic analysis is required for clinical translation.

Profiling Platelet Activation in MPNs Using Flow Cytometry: A Focus on Myelofibrosis

Ann Wang[1], Ryan Collinson[1], Lynne Wilson [1], Kathy Fuller[1], Wendy Erber[12], Matthew Linden[1], Belinda Guo[1]

[1] School of Biomedical Science. University of Western Australia, WA, Australia, [2] PathWest Laboratory Medicine WA, Australia

Myelofibrosis (MF), an aggressive myeloproliferative neoplasm (MPN), is characterized by clonal megakaryocytic dysplasia and progressive bone marrow fibrosis. Despite marked thrombocytopenia in advanced cases, MF carries significant thrombotic risk, suggesting underlying disease-specific qualitative platelet abnormalities. This study investigated platelet activation in MF (n=8) compared to essential thrombocythaemia/polycythaemia vera (ET/PV) (n=28) and non-MPN controls (n=4) under varying stimulation conditions. Platelet activation profiles were quantitatively assessed by flow cytometry following in vitro stimulation with varying concentrations of Thrombin Receptor-Activating Peptide (TRAP) and Adenosine Diphosphate (ADP). Activation status was determined by measuring surface P-selectin (CD62P) expression, indicating α-granule exocytosis during platelet activation, and PAC-1 binding, which detects activated integrin αIIbβ3 (GPIIb/IIIa), a direct readout of inside-out signalling enabling platelet aggregation. At strong agonist concentrations (20 µM TRAP; 20, 2.25, and 0.5 µM ADP), MF platelets demonstrated a hypo-responsive phenotype, displaying 0.8-fold decreases in CD62P expression and 0.9-fold lower PAC-1 binding relative to ET/PV and non-MPN groups. Notably, CD62P+ platelet percentages were significantly lower in MF compared to ET/PV with 20 µM ADP stimulation ($p < 0.05$), with trends observed for 20 µM TRAP ($P < 0.1$) and 2.25 µM ADP ($p < 0.1$). In contrast, low-dose stimulation (1.5 µM TRAP; 0.025 and 0.0009 µM ADP) elicited approximately two-fold higher activation in ET/PV and MF platelets relative to non-MPN controls, suggesting a hyperreactive response, although with a consistent trend of lower activation in MF versus ET/PV across conditions. While inter-patient variability and limited sample size prevented some comparisons from reaching statistical significance, this work provides the first preliminary evidence of platelet exhaustion in MF. It also lays the groundwork for ongoing study into thrombopathic mechanisms in MF, with the potential to inform the development of targeted therapies to mitigate bleeding and thrombotic risks in MF.

F3

Novel Therapy to Promote Recovery for Leukaemia Patients Undergoing Haematopoietic Stem Cell Transplant

Lim. A[1,2], Cheung. LC[1,2], Kotecha. R[1,2,3]

[1]Curtin University, [2]The Kids Research Institute Australia, [3]University of Western Australia

Introduction. Hematopoietic stem cell transplant (HSCT) is one of the treatment options to treat high-risk leukemia patients. Despite modern success, post-transplant complications continue to persist partially due to poor blood stem cell recovery. During HSCT, patients are susceptible to infections that may be life-threatening until the transplanted cells from donors successfully engraft into the host bone marrow (BM). **Problem Statement**. Hence, there is a desperate need to identify novel therapeutic approaches to mitigate the risk of infections for these patients. **Procedures**. OM-85 is an attractive therapy option due to its ability to modulate the immune system to fight off infections and promote blood cell recovery in lethally irradiated mice. Healthy immunocompetent mice were treated daily with OM-85, then immune and haematopoietic stem and progenitor populations (HSPC) in the BM and spleen were enumerated. **Results**. HSC population in the BM increased after 10-days of OM-85 treatment. Extension of OM-85 treatment to 20-days increased multipotent progenitor one and common lymphoid progenitor populations in BM. Additionally, myeloid immune cells in the spleen increased after 20-days. **Conclusions**. Given that OM-85 treatment increased HSPC populations in healthy mice, OM-85 treatment can potentially accelerate haematopoietic recovery post-HSCT, thereby, supporting the development of a functional immune system that can mitigate life-threatening infections in high-risk leukaemia patients.

F4

Efficacy of NAMPT inhibition in Down Syndrome-associated Acute Lymphoblastic Leukaemia

Amelia Dyton[1], Kunjal Panchal[1], Bree Foley[2], Rishi Kotecha[1], Sébastien Malinge[1]

[1]The Kids Research Institute Australia, [2]The University of Western Australia

Leukaemia is the most common paediatric cancer and is the second most common cause of death by cancer in Australian children. Children with Down Syndrome (trisomy 21) have a 20-fold increased risk of developing a particularly aggressive type of leukaemia called Acute Lymphoblastic Leukaemia (DS-ALL). Compared to non-DS individuals, children with DS-ALL have lower overall survival rates, and increased treatment-related mortality, and a higher risk of developing relapse. Therefore, there is an urgent need for better, safer, and more targeted therapies for these children. Our lab recently screened >9000 compounds in unique DS-ALL cell lines and identified nicotinamide phosphoribosyltransferase (NAMPT) inhibitors as one of the most promising families of drugs. Inhibition of NAMPT has been shown to be effective in killing leukaemia cells, including ALL, both *in vitro* and *in vivo*, due to their strong dependence on nicotinamide (NAD+) synthesis, but has never been tested in DS-ALL specifically. Here, we extended this analysis and assessed the efficacy of several NAMPT inhibitors *in vitro*. First, we performed dose/response experiments to calculate the IC50 values for several NAMPT inhibitors in a panel of nine ALL cell lines, including four DS-ALL cell lines uniquely available in our lab. Next, we tested potential synergy between our most promising target, OT-82, and standard-of-care (S.O.C) agents, as well as novel targeted therapies for ALL patients. Finally, we pre-treated ALL cells with NAD+ prior to treatment with OT-82, to confirm the specificity of this compound. We found that *CRLF2*-r ALL cell lines, regardless of them being DS-ALL and non-DS ALL cell lines, were particularly sensitive to NAMPT inhibition with OT-82. Our synergy assays revealed an additive effect between OT-82 and S.O.C drugs (vincristine, dexamethasone, L-Asparaginase) *in vitro*. These pre-clinical findings identify OT-82 as a promising new therapy with the potential for further investigation *in vivo*.

Exploring the mechanistic pathways utilised by aurora kinase inhibitors to induce cytotoxicity in KM2TA-rearranged infant acute lymphoblastic leukaemia cells.

Haima Sankar Jayasankar[1], Taylor Fegurson[1,2], Laurence Cheung[1,2], Rishi Kotecha[2]

[1]Curtin University, [2]The Kids Research Institute

Leukaemia refers to the uncontrolled proliferation of white blood cells and encompasses a group of hematological malignancies. A subtype that is of the highest prevalence in the pediatric population is Acute lymphoblastic leukaemia (ALL), particularly affecting infants of the B-cell lineage. Infant ALL (iALL) results from genetic alterations often from the rearrangement of the KMT2A gene (formerly MLL). Infant patients in particular are associated with poorer prognosis and lower rates of survival. Current therapies involve following chemotherapeutic regimens however studies have shown high risk of relapse and overall low event-free survival rates for iALL patients. Although there has been research into targeted therapies such as inhibiting FLT-3 or proteasome, promising outcomes are very limited and insignificant. An emerging approach to the urgent need for novel therapy involves directly targeting the cell cycle of leukaemic cells. This can be achieved through the inhibition of aurora kinases (AURK), which has been shown to be directly involved in the stages of the cell cycle as mitotic regulators. This targeted disruption of cell cycle will inhibit cell division and promote apoptosis in leukaemic cells. The aim of this project is to understand the mechanistic pathways in which AURK induces this cytotoxicity in our selected KMT2A-r iALL cell lines: PER-910, PER-490, KOPN8. The 3 novel AURK inhibitors that will be investigated are: Alisertib, Hesperadin, and Tozasertib. The efficacy of these AURK inhibitors will be assessed using flow cytometry. The apoptotic outcome from the AURK inhibition will be assessed using Annexin V and Propidium Iodide staining and further validated by detection of Cleaved PARP, Cleaved Caspase and yH2AX. Mitotic cell progression and polyploidy will also be assessed through staining cells for pHH3 alongside Hoechst and Ki-67 intracellular staining. Findings from this project will deepen the understanding of the underlying mechanisms of AURK inhibitor induced cytotoxicity which may improve the development of more effective targeted therapies for KMT2A-r iALL patients.

Identifying Early Treatment Changes in Autoantibody Levels Concordant with Response to Pembrolizumab in Late-stage Melanoma Patients

Rebecca Auzins[1,2], Anna Reid[1,2], Professor Elin Gray[1,2], Dr Pauline Zaenker[1, 2]

[1] Centre for Precision Health, Edith Cowan University, Perth, Australia, [2] School of Medical and Health Sciences, Edith Cowan University, Perth, Australia

Introduction. Predicting a patients' response to treatment is a key component for precision health. Current response rates for pembrolizumab, a treatment for late-stage melanoma, still leaves room for improvement with less than half of all patients responding to the treatment by 12 weeks. Studying how specific autoantibodies change during treatment could lead to earlier tools for measuring treatment response, ultimately improving patient outcomes. **Problem Statement**. We have identified at least one autoantibody that may be suitable for the monitoring of patient treatment response to pembrolizumab. **Procedures**. We analysed a proteomic dataset from 25 late-stage melanoma patients. The analysis focused on statistically significant increases in the abundance of IgA and IgG antibodies (~21,000 of each) between baseline and early during treatment (6-9 weeks) in responders. These candidate markers were subsequently assessed for group-wise differences (responders n=15 vs. non-responders, n=10) using a comparative analysis, then had their performance evaluated as a classification model using a ROC curve. **Results**. A total of 16 IgA and 13 IgG antibodies exhibited a statistically significant difference ($p = <0.05$) in their distribution patterns between the patients that responded well to the treatment (responders) and those who did not (non-responders). A final number of 9 IgA and 10 IgG antibodies displayed a significant performance rating ($p = <0.05$, $AUC = >0.7$). Of these, only one IgA antibody and one IgG antibody had an AUC of 0.8 with a sensitivity of 0.8 and specificity of 0.8. **Conclusions**. Autoantibody titres of ST6GALNAC1 (IgA) and CLIC5 (IgG) were significantly elevated at follow-up (6-9 weeks) for late-stage melanoma patients that responded to anti-PD-1 treatments. This knowledge could provide earlier diagnostic tools for response assessment, ultimately leading to improved patient outcomes. Further analysis is required to validate these markers and confirm their use for predicting treatment response.

Blood-based biomarkers for the detection of melanoma brain metastasis

Désirée Sexauer[1,2]*, Russell J Diefenbach[3,4]*, Lydia Warburton[1,2], Ashleigh Stewart[3,4], Anna L Reid[1,2], Wei Yen Chan[3,4], Jenny H Lee[3,4], Alexander M Menzies[4], Inês Pires da Silva[4], Georgina V Long[4], Pauline Zaenker[1,2], Aaron B Beasley[1,2], Elin S Gray[1,2]†, Helen Rizos[3,4]†

1. Centre for Precision Health, 2. School of Medical and Health Sciences, Edith Cowan University, 3. Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, 4. Melanoma Institute Australia, Sydney

Introduction. Brain metastases in melanoma are typically diagnosed following neurological symptoms or imaging, often at advanced stages. Early detection of asymptomatic brain metastases is crucial, as progression can lead to severe neurological decline, diminished quality of life, and death. **Methods.** This study examined circulating biomarkers, including microRNAs, tumour-associated autoantibodies, and brain-derived cell-free DNA (cfDNA) methylation— from baseline blood samples of melanoma patients with intracranial metastases (intracranial only or concurrent extracranial) and those with extracranial-only metastases, prior to systemic therapy. For cfDNA methylation analysis, 47patients with brain metastases and 97 with extracranial only metastases were included. The microRNA cohort consisted of 125 patients (intracranial = 41, extracranial = 84), with sample size reduced due to haemolysis in 19 samples. Autoantibody profiling was conducted on 95 serum samples; four were excluded due to quality control failure. MicroRNA libraries were prepared using the QIAseq miRNA Library Kit (Qiagen) and sequenced on the Ion Torrent S5 Prime (Thermo Fisher). Differential expression analysis was conducted using edgeR, with significance defined as a fold change ≥1.2 and a false discovery rate (FDR) ≤ 0.001. Validation with TaqMan MicroRNA Assays is planned. Autoantibody biomarkers were identified using HuProts v.4 (~17,000 proteins); IgG markers will be validated on custom arrays. cfDNA methylation was analysed using a custom QIAseq Targeted Methyl Panel of brain-specific CpGs; a refined panel will be developed for validation. **Results**. One microRNA (microRNA-1246) was significantly enriched in patients with brain metastases (log2FC = 1.99, FDR = 0.000021). 203 autoantibodies ($p < 0.01$, log2FC > 1.5), including 49 IgG markers (AUC ≥ 0.7), were elevated in the brain metastasis group. Eight cfDNA methylation markers also showed promise (FDR < 0.05). **Conclusions**. A multi-analyte approach using circulating biomarkers may enable earlier, non-invasive detection of brain metastases in melanoma. A validation cohort is currently under evaluation.

Using Artificial Intelligence to identify Autoantibodies as Biomarkers of Response to Immunotherapy and Immune-related Adverse Events in Cutaneous Melanoma Patients

Shajib Dey[1,2], Elin Gray[1,2], Johnny Lo[4], Syed Afaq Ali Shah[3] †, Pauline Zaenker[1,2] †

1. Centre for Precision Health, 2. School of Medical and Health Sciences, 3. Centre for AI and Machine Learning, School of Science, 4. Mathematical Applications and Data Analytics (MADA), School of Science, Edith Cowan University,

Melanoma is an aggressive skin cancer with increasing incidence and high mortality. Although ICIs targeting PD-1 and CTLA-4 have markedly improved outcomes, only 30–50% of patients achieve durable responses, and up to 30% develop severe immune-related adverse events (irAEs). Existing tissue-based biomarkers such as PD-L1 expression, tumour mutational burden, and tumour-infiltrating lymphocytes show inconsistent predictive accuracy. Autoantibodies (AAbs) offer a minimally invasive, stable readout of systemic immune activity and may overcome limitations of tissue-based markers. This project aims to discover and clinically validate serum autoantibody (AAb) signatures predictive of both clinical response and irAE risk in melanoma patients receiving ICIs, using high-throughput HuProt™ microarrays and AI-driven analysis. Baseline serum from two independent cohorts (n = 192) will be screened on HuProt™ arrays (>21,000 proteins) to profile IgG and IgA AAbs. After data preprocessing (log-transformation, RLM normalisation), supervised machine learning models (random forests, SVM, gradient boosting) with five-fold cross-validation will distinguish responders from non-responders and classify irAE severity (CTCAE v5.0). Model performance will be assessed by ROC AUC, accuracy, precision, recall, and F1 score. Key AAb candidates (log FC > 0.6, $p < 0.05$) will undergo pathway enrichment to interpret immune mechanisms. Anticipated outcomes include AAb panels achieving AUC > 0.8 for response prediction and early irAE detection. Top-ranked AAbs from both IgG and IgA panels will be transitioned into bead-based multiplex immunoassays for clinical validation using longitudinal samples collected at multiple time points. This research will integrate HuProt™ microarray profiling and AI to generate clinically actionable AAb signatures for personalised melanoma immunotherapy. By enabling prediction of both efficacy and toxicity and translating findings into scalable multiplex assays, this project seeks to improve patient stratification, optimise treatment decisions, and reduce irAE-associated risks, ultimately advancing precision oncology for melanoma.

Establishing a Syngeneic Mouse Model of Tumour-Infiltrating Lymphocyte Therapy in Metastatic Melanoma

N. Batohi[1,2,3], H.V. Newnes[2], E. S. Gray[1,3,4], J.A. Nilsson[3,5], J. Waithman[2]

[1]Centre for Precision Health, Edith Cowan University[2]School of Biomedical Sciences, The University of Western Australia, [3]Melanoma Discovery Laboratory, Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, [4]Melanoma Institute of Australia, The University of Sydney, [5]Sahlgrenska Center for Cancer Research, Department of Surgery, Institute of Clinical Sciences, University of Gothenburg and Sahlgrenska University Hospital, Gothenburg, Sweden

Adoptive cell therapy using tumour-infiltrating lymphocytes (TILs) has demonstrated durable responses in patients with advanced melanoma. However, the complexity of human TIL therapy and limited access to patient material constrain mechanistic studies and preclinical optimisation. To address this, we are developing a syngeneic murine model that recapitulates key features of TIL therapy in a controlled setting. Naïve CD8 T cells specific for the herpes simplex virus glycoprotein B (gB) epitope were adoptively transferred into C57BL/6 mice. Mice were subsequently engrafted epicutaneously with B16 melanoma cells genetically modified to express gB. Primary tumours and metastatic lesions were harvested to isolate the T cells residing within them, generating so-called "young" TIL cultures. Initial ex vivo expansion was tested across three different cytokine support conditions. Tumour reactivity was tracked via flow cytometry using CD8, CD45.1, and Vα2markers to quantify expansion of the transferred gB-specific T cells. Preliminary findings show rapid lymphocyte expansion when stimulated with anti-CD3/CD28 in the presence of interleukin (IL)-2 and IL-7. Current efforts are focused on refining culture conditions to limit tumour overgrowth and enhance TIL expansion. This model supports the systematic evaluation of antigen-specific T cell recruitment into the tumour microenvironment and the optimisation of in vitro TIL expansion protocols. Our overarching goal is to adoptively transfer expanded TILs into lymphodepleted, tumour-bearing hosts to assess in vivo anti-tumour efficacy. Ultimately, the successful development of this platform will enable in-depth investigation of TIL biology and provide a valuable tool for testing and refining strategies aimed at enhancing the efficacy of TIL-based immunotherapies.

Boosting Immunotherapy: Leveraging mRNA to Sensitise Paediatric Solid Tumours

Juliet Schreurs[1,2], Wang Li[1,2], Joost Lesterhuis[1,2], Ben Wylie[1,2]

[1]Sarcoma Translational Research Group, The Kids Research Institute Australia,[2]The University of Western Australia

Paediatric solid tumours, including soft tissue sarcomas, often have immunologically "cold" microenvironments, characterized by limited immune cell infiltration and low tumour mutational burden. This limits the effectiveness of immune checkpoint blockade therapies, which have shown success in adult cancers. This project aims to develop an mRNA-based immunotherapy to reprogram the tumour microenvironment and enhance immunotherapy responsiveness. By activating specific immunogenic signalling that can "heat up" these tumours, we aim to boost their sensitivity to immunotherapy. mRNA offers a flexible platform to transiently express immune-activating proteins, allowing for reshaping of the tumour milieu to drive durable anti-cancer responses. We first screened a panel of mRNA candidates encoding immune-stimulatory proteins, both individually and in combination, to assess their ability to prime relevant immune cell types and enhance response to subsequent adjuvant therapies. Lead candidates were validated *ex vivo* using murine bone marrow-derived dendritic cells, showing enhanced activation and maturation in response to stimulation. These preliminary findings demonstrate the immunostimulatory potential of selected mRNA constructs, supporting their progression to in vivo testing in cancer models. This strategy may provide a new therapeutic avenue to sensitise paediatric solid tumours to immunotherapy responsiveness.

Exploring and exploiting the antigenic landscape of mesothelioma for cancer vaccine development

Jessica Boulter[1,2,3], Shaokang Ma[1,2], Ian Dick[1,2,4], Georgia Hosking[1,2,3], Nandini Makwana[1,2], Wen-Shuz Yeow[1,2], Kirti Pandey[5], Pouya Faridi[5], Irina Caminschi[5,6], Anthony Purcell[5], Bruce Robinson[1,2,3,7], Jenette Creaney[1,2,3,7] and Alec Redwood[1,2,4]

[1]Institute for Respiratory Health, [2] National Centre for Asbestos Related Diseases, [3]Medical School, [4]School of Biomedical Sciences, [5]Infection and Immunity Program & Department of Biochemistry & Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, [6]Department of Microbiology & Immunology, Peter Doherty Institute, The University of Melbourne, [7]Department of Respiratory Medicine, Sir Charles Gairdner Hospital

Tumour-specific mutations that lead to the expression of novel, or "neo"-antigens are key targets for personalised cancer vaccines. Neoantigen vaccines show promise in high-tumour mutation burden (TMB) cancers such as melanoma and lung cancer. However, their suitability for low-mutation tumours such as mesothelioma remains unexplored. We sought to comprehensively map the neoantigen landscape of mesothelioma and determine if low-mutation burden cancers express sufficient neoantigens for effective vaccination. To identify potential neoantigens we performed genomic sequencing and immunoproteomics in the AB1-HA mouse mesothelioma model. Predicted neoantigens were tested for immunogenicity by interferon-gamma ELISpot in naïve tumour-bearing mice, and in re-challenged tumour immune mice. Immunogenic neoantigens were then evaluated for efficacy in various vaccine settings. Sequencing identified 1385 non-synonymous single nucleotide variants (nsSNV) and small insertions/deletions (indels), and 8 gene fusions. Of these, 220 nsSNV/indels and 6 gene fusions encoded potential neoantigens. Immunoproteomics identified >20,000 peptides on the tumour cell surface, including 5 derived from nsSNV neoantigens, 3 from tumour-associated antigens (TAA), and 67 from endogenous retroviral elements. In total 440 synthetic peptides were screened for immunogenicity in tumour-bearing and tumour-immune mice. Surprisingly, only 5 neoantigens and one TAA induced detectable T-cell responses. The paucity of responses was not due to poor immunogenicity, as 58% (32/55) of tested neoantigens induced detectable T-cell responses when included in a vaccine. Importantly, neoantigens recognised by T-cells in tumour-bearing or tumour-immune mice were capable of protecting mice from tumour when delivered as a neoantigen vaccine. **Conclusion:** We have tested ~16% of the AB1-HA mutanome and several other classes of tumour antigen, which to our knowledge is one of the most complete neoantigen mapping studies to date. We have observed immunogenicity rates (1.7%) comparable to high-TMB cancers, and most importantly have demonstrated that neoantigen vaccination is feasible in mesothelioma, providing a pathway for future therapeutic opportunities.

The Effects of Low-Dose Radiotherapy on Tumour-Associated Macrophage Polarisation in a Murine Mesothelioma Model

Authors: Mackenzie Lighton[1,2], Faith Hartley[2,3], Rebecca D'Alonzo[2,4], Alistair Cook[1,2], Martin Ebert[4]

1. School of Biomedical Sciences University of Western Australia (UWA) Nedlands Australia. 2. National Centre for Asbestos Related Diseases Institute for Respiratory Health UWA Nedlands Australia. 3. Medical School UWA Perth Australia. 4. School of Physics Mathematics and Computing UWA Perth Australia.

Mesothelioma is a fatal thoracic cancer primarily caused by asbestos exposure. Immune checkpoint inhibitors (ICIs) are the current first line of treatment for pleural mesothelioma patients. However, most patients exhibit primary or acquired resistance to ICIs, which may be partly due to an immunologically 'cold' tumour microenvironment (TME). Tumour-associated macrophages (TAMs) represent a significant portion of tumour-infiltrating leukocytes. Throughout tumour development, the phenotype of TAMs shifts from anti-tumour ('M1-like') to tumour-supportive ('M2-like'), accelerating disease progression. Preliminary data suggests that low-dose radiotherapy (LDRT) can influence the polarisation of TAMs, shifting these cells towards the tumoricidal 'M1-like' phenotype, which may improve the efficacy of ICI treatment. This study aims to validate preliminary gene expression data, which suggests that LDRT shifts macrophage polarisation towards the 'M1-like' phenotype. We hypothesise that AB1-HA tumours subjected to irradiation of 2 Gy x 5 fractions will express a higher proportion of 'M1-like' markers compared to sham-irradiated tumours. Additionally, irradiated AB1-HA tumours harvested 1 day following final irradiation will express a greater level of 'M1-like' markers compared to irradiated tumours harvested 6 days following final irradiation. The AB1-HA mesothelioma cell line will be used to establish tumours in BALB/cJAusBP mice. Tumours will be treated with either LDRT (2 Gray x 5 fractions) or will be sham irradiated. Mice will be culled at 1 and 6 days following final irradiation. Using an optimised antibody panel, spectral flow cytometry will be used to analyse and quantify specific macrophage markers present in tumour samples at the two time points. The findings of this study will provide insight into the effects of LDRT on TAM polarisation. This insight may inform the development of combination therapies between LDRT and ICIs, and biomarker strategies for patient selection to improve the efficacy and safety of treatment options for pleural mesothelioma patients.

A lentiviral pseudovirus based system for determining neutralizing antibodies to SARS-CoV-2

Modiri Tau[1], Connie Jackaman[1], Delia Nelson[1], Pieter Eichhorn[1], Rina Wong[1,2], Padraig Strappe[1,2,3]

[1]Curtin Medical Research Institute, Curtin Medical School, [2] School of Medical and Health Sciences, Edith Cowan University, [3] Charles Darwin University Menzies Medical Program, Charles Darwin University

The continued evolution of SARS-CoV-2 variants, especially Omicron and its subvariants, from the previously circulating strains, XBB and JN.1 to the currently circulating NB.1.8.1 remains a continuous world-wide problem to COVID-19 vaccine strategies due to their high transmissibility and immune evasion abilities. The spike (S) protein, which is crucial for viral infectivity by attaching to the host angiotensin-converting enzyme II (ACE2) receptor, is targeted by most of the vaccines. The presence of mutations within the S protein's receptor-binding domain (RBD) in Omicron variants impair the binding of neutralizing antibodies (nAbs), leading to a reduction in vaccine neutralization efficacy. The continuous monitoring of nAbs against the current Omicron variants is critical in providing guidance in future vaccine policies and public health interventions. This study utilizes a safe and reliable lentiviral pseudovirus assay, suitable for BSL-2 laboratories, to assess humoral immunity. We successfully generated a 293T-hACE2 cell line and SARS-CoV-2 pseudotyped lentiviruses, B.1.617.2 (Delta), B.1.1.529 (early Omicron) and XBB.1.5 (Omicron variant). Analysis of transduction efficiency of the generated pseudoviruses based on Green Fluorescent Protein (GFP) and firefly luciferase expression showed a high transduction by SARS-CoV-2_XBB.1.5 pseudovirus. A neutralization assay with SARS-CoV-2 monoclonal antibodies revealed a more than 50% inhibitory dilution (ID50) at a 1:27 dilution for four out of five of the antibodies tested demonstrating some neutralizing activity against the XBB.1.5 pseudovirus. However, this activity was observed only at high antibody concentrations, highlighting the potential for reduced efficacy of antibodies developed against the original strain in their ability to neutralize Omicron variants. The lentiviral pseudovirus assay is a suitable platform for evaluating nAb levels. The next steps will involve generating pseudoviruses for the currently circulating Omicron variants, to assess neutralizing antibody levels across different age groups. This research provides valuable insights that could inform age-specific vaccine strategies and policy decisions.

Evaluation of the Immune Microenvironment Post Brain Surgery

Handley, Demi[1]; Johnson, Piyusha[1]; McDonnell, Alison[1,2]; Endersby, Raelene[1,2] and Dewdney, Brittany[1,2]

Affiliations for all authors. [1]The Kids Research Institute Australia, [2]Centre for Child Health Research, University of Western Australia

Introduction: Brain cancer is the second most common paediatric cancer and accounts for the highest number of cancer-related deaths in children. Surgical resection is a key treatment step for most paediatric brain cancer patients. In other cancers, surgery has been shown to trigger a coordinated immune response as part of tissue repair. Presently, this remains relatively unexplored in brain cancer; therefore, the landscape of the immune microenvironment and its impact on brain tumour growth and response to treatment remains unclear. **Problem Statement**: This study aimed to characterise the immune landscape of the brain following surgery, using a non-tumour model, to better understand how the post-operative immune response might shape outcomes in paediatric brain cancer. **Methods**: Using a non-tumour surgical model to mimic brain surgery, we evaluated the brain-immune microenvironment in C57BL/6 mice. Immunohistochemistry and spectral flow cytometry were used to identify immune cell populations in the mouse brain at days 1, 3, 5, 7, 14 and 28 post-surgery. **Results:** We have preliminary evidence demonstrating a distinct spatiotemporal shift in infiltrating immune populations in the healthy adult mouse brain. Most notably there is a transient influx of myeloid-derived cells to the surgical site, including neutrophils, monocytes, and dendritic cells. In addition, resident microglia and astrocytes show persistent, long-term activation surrounding the injured area. **Conclusion:** This pilot study shows how the brain-immune microenvironment is modulated post-surgery. We hypothesise that immune features driving brain wound healing will differ significantly in the context of brain tumour resection surgery, and again in the paediatric mouse brain, which we aim to explore in the future. Identifying key immune populations and their functions will reveal clinically relevant immunotherapy targets that can be used in parallel with the wound healing response to enhance anti-cancer effects in paediatric brain cancers.

Therapeutic targeting of airway remodelling and inflammation in allergen-sensitised mice

Aaron R. Gomes[1,2], Luke Berry[1], Emma Catchpole[2], Alexander N. Larcombe[1,3], Patricia Agudelo-Romero[1,2], Peter B. Noble[2], Anthony Kicic[1,2,3], Stephen M. Stick[4], Thomas Iosifidis[1,2,3]

[1]The Kids Research Institute Australia, [2]The University of Western Australia, [3]Curtin University of Technology, [4]Child and Adolescent Health Services

Introduction. Children with persistent wheeze are vulnerable to lung function decline and early asthma diagnosis. Defective airway epithelial cell (AEC) repair is implicated in airway remodelling, inflammation, and asthma severity. We previously showed *in vitro* that defective AEC repair correlates with recurrence of childhood wheeze and is correctable by administration of celecoxib (CXB) or its non-COX2-inhibiting analogue, 2,5-dimethyl-celecoxib (DMC). **Problem Statement**. Here, we assessed therapeutic efficacy of CXB and DMC *in vivo* using a mouse model of house dust mite (HDM)-induced airway sensitization, hypothesising both compounds would attenuate airway remodelling and inflammation compared with vehicle-control. **Procedures/Data/Observations**. Eight-week-old BALB/c mice (21M+21F) were intranasally inoculated with 25µg HDM protein daily for 14 days. On days 11–14, mice received daily intranasal treatments of vehicle (n=15), CXB (n=12), or DMC (n=15), ≥3 h post-HDM. On day 15, mice were euthanised, lungs formalin-fixed-paraffin-embedded for histology, and blood collected for ELISA on total serum IgE. Lung sections were scored (1=normal/2=mild/moderate/3=severe) for epithelial denudation, goblet cell hyperplasia, inflammation, and collagen density, with cumulative severity scores (CSS) calculated and reported as median [IQR]. IgE values were log-transformed, and reported as mean±SD. Data were analysed by 2-way ANOVA. **Results**. No sex or treatment effects were observed on CSS, despite a trend towards lower CSS in DMC-treated mice (7.2 [6.7–7.8]) compared with vehicle (7.7 [7–8.4]), and CXB (8.6 [8.2–9]) groups. In males, IgE was lower between DMC- (2.7±0.7ng/mL) and vehicle-treated mice (3.2±0.6ng/mL; p=0.04). There was no effect of DMC on IgE in female mice and no effect of CXB in either sex (p=0.57). **Conclusions**. Pronounced CSS and IgE confirm a robust to severe response post-HDM exposure. While some evidence supports therapeutic effects (particularly DMC) on airway disease, further studies are required to evaluate their potential for targeting allergic asthma in a less extreme exposure model.

Live-Cell Biosensors Reveal Unique Receptor Pharmacology Relevant to Chronic Inflammatory and Fibrotic Diseases

Authors: Henry G Purbrick[1,2,3,4], Elizabeth KM Johnstone[1,2], Carl W White[4], Robert Shepherd[4], Kevin DG Pfleger[1,4].

Affiliations: [1]Molecular Endocrinology and Pharmacology, Harry Perkins Institute of Medical Research. [2]School of Biomedical Sciences, The University of Western Australia. [3]CSIRO Health & Biosecurity. [4]Dimerix Biosciences Pty Ltd.

Introduction. Chronic inflammation and organ fibrosis underpins much of the morbidity of many common diseases, such as chronic-obstructive pulmonary disease, diabetes, stroke, cirrhosis, heart disease and chronic kidney disease. These diseases cause 18% of all mortality worldwide (1). There is clearly an unmet need in controlling the pathophysiological inflammatory axes of these diseases effectively. G protein-coupled receptors (GPCRs) are prominent targets of existing therapeutics, owing to their cell membrane expression, and profound potential for modulating physiology. Some GPCRs are shown to influence the function of other GPCRs through heteromerisation, presenting new opportunities for pharmacological intervention. **Problem Statement**. To identify and characterise novel GPCR heteromers involved with chronic inflammation and/or fibrosis. **Procedures/Data/Observations**. Receptor-HIT is a proprietary assay format to identify receptor-receptor proximity. The β-arrestin2 recruitment bioluminescence resonance energy transfer (BRET) HIT assay was conducted to screen combinations of GPCRs with relevance to chronic inflammation and fibrosis. From this screen, potential lead candidates proceeded toward pharmacological characterisation using BRET-based biosensors. These include sensors for GPCR intracellular trafficking, G protein activation and second-messenger generation. **Results**. Receptor-HIT identified multiple novel heteromeric candidates. A selected candidate demonstrated distinctive pharmacology dependent on protomer co-stimulation with endogenous agonists. An asymmetrical perturbance to internalisation was observed upon co-stimulation of protomers with the BRET trafficking sensor. The G protein activation BRET sensor reveals a similar effect on upon co-stimulation, with this effect extending to a complimentary second-messenger BRET sensor. **Conclusions**. This project has identified a novel candidate with pharmacology consistent with the criteria to classify a GPCR heteromer, which will undergo further scientific and commercial validation.

Determinants of phage stability during nebulisation for treating antimicrobial resistant lung infections

Authors: Rohan Flint[1,2], Daniel R Laucirica[2], Mark P Nicol[3,4], Anthony Kicic[2,5,6,7]

[1]School of Biomedical Sciences, [2]Wal-yan Respiratory Research Centre, The Kids,[3]The Marshall Centre for Infectious Diseases Research & Training, School of Biomedical Sciences, [4]Division of Infection & Immunity, Faculty of Health and Medical Sciences, The University of Western Australia, [5]Dept of Respiratory and Sleep Medicine, Perth Children's Hospital, [6]Centre for Cell Therapy & Regenerative Medicine, School of Medicine & Pharmacology, The University of Western Australia and Harry Perkins Institute of Medical Research, [7]School of Population Health, Curtin University

Nebulised phage therapy is an emerging treatment for antimicrobial resistant lung infections; however, nebulisers can reduce phage viability, with a <1 log reduction considered acceptable for therapeutic use. We evaluated morphological and genomic characteristics of phages as indicators of activity loss during aerosolisation with two commonly used nebulisers, hypothesising phages with longer tails would have greater viability loss. Phages targeting *Pseudomonas aeruginosa* were individually nebulised at a titre of 10^8 plaque forming units/mL using both a vibrating-mesh and jet nebuliser. Phage morphotype was determined by whole genome sequencing and transmission electron microscopy (TEM) (myovirus n=11, jumbo myovirus n=10, siphovirus n=14, and podovirus n=14). Pre and post nebulisation titres were assessed via plaque assay to show loss of phage activity, and damage to phage virions was visualised using TEM. Mesh nebulised phages all exhibited acceptable titre loss for therapeutic use (0.07 – 0.96 log loss), with no significant differences between morphotypes. In contrast, jet nebulisation resulted in robust decreases in viability for 40/49 phages (0.97 – 3.32 log loss), with significant differences between morphotypes. Notably, short-tailed podovirus phages had significantly less loss of activity than other morphotypes ($p<0.01$). Variability in log loss was also observed between different genera within single morphotypes. Significant correlations between log loss and phage genome size and tail length were observed for jet nebulised phages ($p<0.01$). TEM images are currently being analysed, but we expect to see more damaged virions in lower titre post-nebulisation samples. Results indicate that loss of phage activity during nebulisation is strongly influenced by nebuliser type, and phage genome size and tail length also dictate susceptibility to viability loss. These factors could affect treatment efficacy of nebulised phage therapy for antimicrobial resistant lung infections. Future work will trial phage formulations with different buffers and excipients to minimise phage loss during nebulisation.

An Investigation of the Seroprevalence of Ross River Virus in Malaysia using Plaque Reduction Neutralization Tests

George Lines[1], A/Prof Allison Imrie[1], Dr Megan Lloyd[1] Dr Alice Michie[2], Dr Balvinder Gill[3]

The University of Western Australia [1], New South Wales Pathology [2], Ministry of Health Malaysia [3]

Ross River Virus (RRV) is an arthritogenic alphavirus endemic to Australia and Papua New Guinea that has also circulated internationally during the 1979 Pacific Islands epidemic. Due to easily misdiagnosed symptoms, a lack of surveillance, and low vector specificity, there is potential for unrecognized RRV transmission in the Indo-Pacific region. As multiple alphaviruses cocirculate in Southeast Asia it is necessary to use a highly specific assay that can accurately differentiate between antigenically similar antibodies to identify past infections. In this study, a plaque reduction neutralization test (PRNT) was optimized to detect RRV neutralizing antibody in human serum and distinguish between other regional alphaviruses, Sindbis virus (SINV) and Argyle virus (ARGV). The seroprevalence of RRV, SINV, and ARGV neutralizing antibody was assessed from 239 archival serum samples collected from Malaysia in 2010. The PRNT identified five samples that could neutralize RRV but to a magnitude lower than the reference antiserum. Neutralizing antibody present in these samples is likely to be specific for CHIKV as it is prevalent in Malaysia and is cross-reactive with RRV. The PRNT also identified five samples with ARGV neutralizing antibody which corresponds with suspected incidental ARGV infections in the Malaysian population. However, in the absence of reference antiserum, estimates on antibody specificity are not definitive. This study optimized an assay able to distinguish between antigenically similar alphaviruses and identified evidence of alphavirus cocirculation in Malaysia. Neglected alphaviruses should be considered in future arbovirus surveillance efforts across the Indo-Pacific.

Investigating Commensal-Dependent Antibiotic Resistance in *Streptococcus pyogenes*

Bonnie L. Hyatt[1], M. Kalindu D. Rodrigo[1], Janessa Pickering[1], August G. Mikucki[1], Timothy C. Barnett[1,2]

[1] Wesfarmers Centre of Vaccines and Infectious Diseases, The Kids Research Institute, [2] The Marshall Centre for Infectious Disease Research and Training, School of Biomedical Sciences, University of Western Australia

Research Topic and Objectives: Previous studies have suggested that current phenotypic *in vitro* antibiotic susceptibility tests produce misleading results because there is growing evidence that bacteria utilise factors of the host environment to bypass antibiotic action. In this study we have investigated the ability of oropharyngeal commensal bacteria (natural residents of the host environment) to rescue *Streptococcus pyogenes* from clinically relevant antibiotics. **Methods:** Epsilometer tests (Etest), antibiotic broth microdilution assays and checkerboard assays supplemented with host factors (e.g. metabolites and commensal supernatant) were used to identify phenotypic changes in antibiotic susceptibility. A modified Etest utilising a commensal underlay culture was developed to investigate commensal-pathogen cross-protection. **Results:** Supplementation of growth media with commensal supernatant did have considerable effects on antibiotic susceptibly profiles. *S. pyogenes* isolates that appeared susceptible to penicillin on standard antibiotic susceptibility test media (Mueller Hinton Agar) became resistant to penicillin when the same media included enzymes/metabolites secreted by oropharyngeal commensals. **Significance:** This study has identified that commensal strains can affect antibiotic susceptibility of an important pathogen and may explain why *S. pyogenes* infections relapse. Future work will help to develop *in vitro* antibiotic susceptibility tests that can account for host factors and therefore prevent antibiotic treatment failure.

Acute Rheumatic Fever – A Systemic Strep A Infection?

Vanessa Tenaglia[1,2], Jasreen Kular[2], Jua Iwasaki[1], Wenna Lee[1], Timothy C. Barnett[1,2]

[1]Wesfarmers Centre of Vaccines and Infectious Disease, The Kids Research Institute Australia, [2]School of Biomedical Sciences, University of Western Australia, Perth, Australia

Introduction. *Streptococcus pyogenes* (Strep A) is a common cause of sore throats. Acute rheumatic fever (ARF) is a post-infection complication of a Strep A sore throat that results in the generation of autoreactive immune cells and antibodies. Recurrent ARF can cause permanent heart valve damage, known as rheumatic heart disease. The exact mechanism by which Strep A triggers ARF remains unclear. The most widely accepted theory is molecular mimicry, where homology between Strep A and human antigens leads to loss of immune tolerance and autoimmunity. However, this theory has notable limitations. **Problem statement**. To address gaps in the molecular mimicry model, this project investigates an alternative mechanism for ARF pathogenesis. We hypothesise that ARF may be triggered by systemic Strep A infection which is able to account for the symptoms observed in ARF. We developed a primary cell infection model to assess the ability of Strep A strains to infect primary epithelial and endothelial cells from different infection sites, examining potential strain-specific differences. **Procedures/Data/Observations**. We tested multiple *emm* types (strains) of Strep A on commercially sourced primary human tonsil epithelial cells and endothelial cells from the aorta, aortic valve, microvasculature, and lymph node. Infections were assessed at 3 hours post-inoculation. Adherence and invasion were quantified by viable count assays to determine the percentage of the initial inoculum recovered. **Results**. Strep A strains were able to infect both epithelial and endothelial cells, with marked strain-specific variation. Tonsilitis-associated *emm* types showed approximately 6-fold higher attachment to tonsil epithelial cells compared to endothelial cells. Attachment of ARF-associated strains to endothelial cells was approximately 7-fold higher than tonsilitis-associated strains. **Conclusions**. ARF-associated Strep A strains exhibit endothelial cell tropism compared to tonsillitis-associated strains, suggesting the presence of distinct virulence factors or infection mechanisms that could potentially contribute to the development of ARF.

Epigenetic Regulation of Horizontal Gene Transfer in *Mesorhizobium japonicum* R7A

Dávid Szabó[1,2], Callum Verdonk[1,2,3,4], Tahlia Bastholm[1,2], and Joshua Ramsay[1,2]

[1]Curtin Medical Research Institute, Curtin University; [2]Curtin Medical School, Curtin University; [3]Center for Crop and Disease Management, Curtin University; [4]School of Molecular and Life Sciences, Curtin University

Mesorhizobium japonicum R7A is a strain of soil-dwelling bacteria capable of forming a symbiotic relationship with legumes in which they convert atmospheric nitrogen to ammonia. The symbiosis genes enabling this interaction are carried on a chromosomally integrated mobile genetic element called ICE*Ml*Sym^{R7A}, which is capable of excising and transferring to non-symbiotic mesorhizobia in the soil. The conjugative transfer of ICE*Ml*Sym^{R7A} is activated by quorum sensing, a mechanism of population-level gene regulation that uses membrane-diffusible signaling molecules called autoinducers. Despite all R7A cells possessing the means to transfer ICE*Ml*Sym^{R7A}, only a small (~2%) subpopulation called 'R7A*' participate in conjugation and quorum sensing. These cells are activated for transfer by an intricate epigenetic switch managed by the DNA-binding proteins QseC and QseC2 and the non-coding antisense transcript *asqseC*. Despite detailed characterisation of this system, the precise molecular events causing R7A cells to enter the R7A* state remain elusive. Recent data from 5'ONT-cappable seq, a technique used to finely map transcription start and termination sites, have highlighted key differences between *qseC2* transcripts in R7A and R7A* cells. Additionally, x-ray crystallography and western blot analyses have provided new insights into the structure and function of the QseC protein. This research has the potential to greatly progress our understanding of epigenetic gene regulation in bacteria and the influence of horizontal gene transfer on prokaryotic evolution. Furthermore, a more complete understanding of this system may have translational applications in the fields of synthetic biology and biotechnology.

A Network of Non-Coding RNAs Controlled by the *Mesorhizobium* Quorum Sensing System Bind an Array of mRNAs Throughout the *Mesorhizobium japonicum* R7A Genome

Lyam Sbrana[1], Tahlia Bastholm[1,2], Jai Tree[3], Daniel Mediati[4], Joshua Ramsay [1] [1]Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University,, Australia [2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, United States[3]School of Biotechnology and Biomolecular Sciences, University of New South Wales, [4]Australian Institute for Microbiology and Infection, University of Technology Sydney

Mesorhizobium spp. are soil bacteria found throughout Australian soils. They form symbiotic relationships with many species of legumes, including crop legumes such as *Cicer arietinum* (chickpea). The symbiotic relationship involves the formation of nodules within the roots of the legume from which *Mesorhizobium* spp. fix atmospheric nitrogen. Successful formation of these nodules requires a population density threshold to be crossed. To determine when this threshold has been crossed, *Mesorhizobium* spp. utilise a cell-cell chemical signalling system called quorum sensing (QS). QS systems involve the production of auto-inducing molecules called *N*-acyl-homoserine lactones (AHLs). One of the QS systems utilised by *Mesorhizobium* spp. is the recently identified *Mesorhizobium* quorum-sensing (MQS) system. In this system, the AHL synthase MqsI and the crotonase-family enzyme MqsC synthesise the AHL 2,4-trans-C12-HSL at a basal level, making its concentration a function of cell density. Once AHL concentration passes a threshold, it starts being bound by MqsR, a transcriptional regulator which, when liganded, binds to an inverted repeat, the *mqs*-box, upstream of *mqsI,* activating its expression. MqsR also binds to seven other *mqs*-boxes upstream of seven non-coding RNA (ncRNAs) genes, *mqsRNA1-mqsRNA7*. These ncRNAs are variously conserved throughout the *Mesorhizobium* genus, with the most conserved, *mqsRNA7*, being found in 207 of 214 *Mesorhizobium* genomes, while the least conserved, *mqsRNA1*, is found in just 12 of the same genomes. Cross-linking ligation and sequencing of hybrids (CLASH) was used to identify RNA-RNA interactions in the transcriptome of *M. japonicum* R7A. 5'ONT-cappable-seq was used to identify the precise transcription start sites and the approximate transcription termination sites of most transcripts of *M. japonicum* R7A and *M. ciceri* CC1192. Of the nearly 15,000 RNA-RNA interactions identified by CLASH in *M. japonicum* R7A, 233 were between *mqsRNAs* and mRNAs. These interactions were further categorised by the location that the *mqsRNA* bound to its target.

Targeting stress in Alzheimer's disease: Evaluating mineralocorticoid receptor modulators and gene variants on microglial function

Amy O'Keeffe[1], Marnie Petruccci[1], Ayeisha Milligan-Armstrong[1], David Groth[1], Giuseppe verdile[1,2].

[1] Curtin Medical School and Curtin Medical Research Institute, Faculty of Health Sciences, Curtin University [2] School of Medical and Health Sciences, Edith Cowan University

Chronic stress has been associated with promoting neurodegenerative diseases through dysregulating the Hypothalamic-Pituitary-Adrenal (HPA) axis and increasing cortisol levels. High cortisol levels have been observed in Alzheimer's disease (AD) patients and promotes the progression of AD pathology. The cellular action of cortisol is mediated by the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), with MR being involved in setting the HPA-axis activation threshold. In addition, studies have revealed the importance of the MR in neuronal cells. However, little is known about its role in microglia which are thought to become maladaptive in AD. Thus, the project aims to assess the impact of modulating MR in microglia. Human microglia cell-line (HMC3) were treated with MR agonist, Fludrocortisone, or MR antagonist, Spironolactone and gene expression was assessed through RNA sequencing. These molecules led to differential expression of several genes, including some related to stress responses, neuroinflammation, and AD. To confirm a major role for the MR in microglia function, current work is assessing the effects of combing MR and GR modulators, as well as establishing HMC3 models of MR KO. The next steps are to express and evaluate the effect of MR haplotypes that are known to alter MR activation and assess their impacts on microglial function via morphology, phagocytosis, migration, and cytokine production analyses. Overall, the findings from this study will provide novel insights into the role of the MR in microglial function and have implications in targeting this receptor in neurodegenerative diseases.

Cellular and Behavioural Late Effects of Irradiation in a Juvenile Preclinical Model

Helena Bertazzo[1], Annabel Short[1], Jamie Beros[2], Raelene Endersby, Jennifer Rodger, Chris Butler

[1]Brain Tumour Research Team, The Kids Research Institute Australia, [2]School of Human Sciences, The University of Western Australia

Introduction. Brain cancer in children disrupts critical development, and its harsh treatments have long term side effects, greatly diminishing quality of life in adulthood. As over 80% of paediatric brain cancer patients survive, improving survivor outcomes has become a research priority. **Problem Statement**. Radiotherapy is particularly associated with neurocognitive deficits, and disrupts cellular processes, leading to late effects prevalent many years after treatment. However, preclinical testing does not routinely assess treatments for late effects. We hypothesised that structural and functional irradiation effects in our pre-clinical model would mimic those in children, allowing late-effects screening. We further hypothesised that increased synaptic density was the cause of cortical expansion seen after fractionated radiotherapy, revealing a possible tissue sparing mechanism. **Procedures**. Juvenile mice were treated at postnatal day (P) 16 with either a single dose of 8 Gy whole-brain irradiation or a mathematically equivalent fractionated dose of 18 Gy (9 × 2 Gy daily fractions). Control mice received either a single or 9x cone-beam computed tomography (CBCT) scans. At P63, mice underwent a behavioural battery associated with brain areas demonstrating volume changes after radiotherapy, assessing cognition, olfaction and social behaviours. Brains were harvested for Golgi staining to investigate dendritic spines as a marker of synaptic density, and for immunofluorescence synaptic staining. **Results**. Fractionated radiotherapy during early life induced neurocognitive deficits in mice akin to those observed in patients. Radiotherapy caused impairments on a hippocampal-dependent spatial memory task and preliminary data demonstrates a significant interaction between radiation and dosing schedule on sociability in a cortex-dependent task. We further expect to see increased synaptic density in mice who received fractionated irradiation. **Conclusions**. As behavioural effects seen in this model are similar to those seen in paediatric brain cancer survivors, this model shows promise to screen for late effects in future treatments.

Developing a Patient-centric Humanised Mouse Model of Congenital Myopathy

Darcy Pirotta[1,2], Omar Elaskalani[2,3], Joshua Clayton[1,2], Gina Ravenscroft[1,2], Raelene Endersby[2,3], Rhonda Taylor[1,3]

[1]Harry Perkins Institute of Medical Research, [2]The University of Western Australia, [3]The Kids Research Institute

Introduction. This project aims to develop a novel humanised mouse hindlimb model that can be customised with the engraftment of patient-derived muscle progenitor cells. This model is novel in that engraftment is performed in neonatal mice without irradiation or muscle damage. Our goal is to capitalise on the naturally maturing musculature of young mice to overcome critical issues with myotube immaturity in existing cell culture models. **Problem statement.** Congenital myopathies are a group of muscle disorders that cause severe mobility loss and difficulties with breathing and swallowing. Despite extensive pre-clinical investigation of novel therapeutics, no curative treatments have been approved. To develop targeted treatments we need tractable, patient-centric disease models that retain the human-genomic context. **Methods.** Healthy and patient-derived induced pluripotent stem cells (iPSCs) were transduced with eGFP and luciferase reporters, then differentiated to skeletal muscle progenitors (iPS-SkM). Fusion index of iPS-SkM was assessed in 2D culture, and myogenic gene expression was characterised using rt-qPCR. Healthy iPS-SkM were injected into the hindlimbs of postnatal day-three NOD/Rag mice. Luciferase assays were performed at week three and eight post-injection to assess cell survival *in vivo*. At week eight, muscles were harvested, snap-frozen and cryosectioned. We performed H&E staining and immunofluorescence for human-specific lamin A/C and dystrophin to evaluate engraftment and fibre formation. **Results**. Healthy and patient-derived iPS-SkM lines resemble embryonic muscle stem cells in gene expression and have a fusion index >50%. We demonstrate engraftment and survival of healthy human iPS-SkM cells eight weeks post-injection into the hindlimbs of neonatal mice, as well as their capacity to form humanised muscle fibre bundles. **Conclusions**. Formation of humanised muscle fibres in NOD/Rag hindlimbs provide proof of concept for a humanised mouse hindlimb model. Further study is required to optimise engraftment, characterise humanised muscle fibres and to assess feasibility with patient derived iPS-SkM.

Role of Gut Microbiota in Alzheimer's Disease: Insights into Physical Activity

Lahiru Perera[1,2], Ian J. Martins[1,2], Favil Singh[3], Ralph N. Martins[1, 2], W.M.A.D. Binosha Fernando[1,2,]
w.fernando@ecu.edu.au

1 Centre of Excellence for Alzheimer's Disease Research & Care, School of Medical and Health Sciences, Edith Cowan University, 2 Alzheimer's Research Australia, Ralph and Patricia Sarich Neuroscience Research Institute,, School of Medical and Health Sciences, Edith Cowan University,

Introduction Alzheimer's disease (AD), characterised by cerebral amyloid-β (Aβ) and tau accumulation, is increasingly linked to alterations in gut microbiota (GMB). These changes include an increased Firmicutes/Bacteroidetes ratio, loss of anti-inflammatory taxa, and reduced short-chain fatty acid (SCFA) production, which may influence AD pathogenesis via the gut–brain axis. Physical activity (PA) is known to beneficially modulate GMB composition, yet its role in AD remains underexplored. **Procedures** A comprehensive literature search was performed using Google Scholar and PubMed. The search strategy combined keywords and Boolean operators: "Alzheimer's Disease" AND "Gut Microbiome" OR "Gut Microbiota" AND "Physical Activity" OR "Exercise" AND "Functional Pathways". Inclusion criteria were: human or animal studies reporting GMB composition in AD, studies evaluating the effects of PA on microbiota, and articles published in English. Exclusion criteria included reviews without primary data, and studies not reporting microbiota outcomes. Data extracted included study design, population characteristics, microbiota profiling methods, PA type and duration, and reported changes in microbial taxa or functional pathways. **Results** Across human and animal studies, AD was associated with significant microbial dysbiosis characterised by increased abundances of pro-inflammatory genera, Escherichia and Shigella alongside reduced levels of Lactobacillus and Bifidobacterium. Functional pathway analyses revealed reduced SCFA biosynthesis capacity and lower level of genes expression. PA interventions ranging from moderate aerobic exercise to endurance training consistently enhanced microbial α-diversity, enriched butyrate-producing taxa including *Faecalibacterium prausnitzii, Roseburia,* Coprococcus, Lachnospiraceae, Clostridiales, and elevated the abundance of *Akkermansia muciniphila*, improved intestinal barrier integrity. Animal studies further demonstrated that PA increased colonic SCFA concentrations, downregulated inflammatory markers, and reduced microglial activation. These changes reduced neuroinflammatory signalling. **Conclusion** PA may help restore microbial homeostasis, increase neuroprotective metabolite production, and attenuate neuroinflammation in AD, representing a promising non-pharmacological approach to slow disease progression. Further longitudinal studies are needed to establish causal pathways and optimise microbiota-targeted strategies in AD management.

Characterisation of peripheral anti-cN1A-reactive B cells in Inclusion Body Myositis.

N. Slater[1], Kelly Beer[1, 2], Annik Panicker[1, 2], Abha Chopra[1], Merrilee Needham[1, 2, 3, 4], Jerome Coudert[1, 2, 3, 5]

1Personalised Medicine Center, Murdoch University, Murdoch, WA 2Perron Institute for Neurological and Translational Science, University of Western Australia, Nedlands, WA 3School of Medicine, University of Notre Dame Australia, Fremantle, WA 4Department of Neurology, Fiona Stanley Hospital, Murdoch, WA5RESTORE Research Center, Université deToulouse, CNRS, INSERM, EFS, 31400, Toulouse, France

Inclusion Body Myositis (IBM) is a progressive muscle disease in adults, characterised by selective muscle weakness and atrophy. IBM pathogenesis involves inflammatory infiltrates, muscle degeneration, and mitochondrial dysfunction. Autoantibodies against cytosolic 5'-nucleotidase 1A (cN1A) are detected in approximately 50% of patients. Seropositivity correlates with more severe disease and reduced survival, though mechanisms remain unclear. Currently there are no effective therapies for IBM. Our goal was to characterise the role anti-cN1A-producing B cells in IBM pathogenesis in order to gain insights into disease mechanisms and to inform future therapeutic strategies. We developed a fluorescence-activated cell sorting (FACS) protocol to isolate circulating cN1A-specific B cells. B cell receptor (BCR) sequences from individual cells were obtained using targeted Illumina MiSeq protocol and analysed using MiXCR and R. For comparison, healthy control BCR repertoires were downloaded from the European Nucleotide Archive. We analysed 221 cN1A-reactive B cells expressing productive BCR from three IBM patients. Clonal expansion within individual patients was evident, with some clones represented by up to 52 identical cells. Two CDR3 regions with identical or highly similar (up to 70% identity) sequences were shared between IBM patients, and occasionally also found at low frequencies in healthy controls. Both sequences were derived from variable light chains. Our findings support antigen-specific expansion of B cells in IBM, rather than non-specific bystander activation. Importantly, we highlight a potential role for the BCR light chain in antigen recognition - an element often overlooked in B cell repertoire studies. Due to the high clonal diversity of cN1A-reactive B cells, selectively targeting these cells may be challenging as a therapeutic strategy; however, this limitation could potentially be overcome by high-throughput, personalised medicine approaches.

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Investigating the Biodistribution of Morpholino Oligomers in the Central Nervous System of Mice

Ianthe Pitout[1], Vanja Todorovski[1,2], Leah Attwood[1], Alanis Lima[1] and Susan Fletcher[1,2]

[1]Personalised Medicine Centre, Murdoch University, [2]University of Western Australia

Antisense oligonucleotide (AO) drugs are single-stranded synthetic RNA molecules designed to modulate the expression of target genes and offer unprecedented precision in therapeutics to treat some of the worst diseases afflicting humanity. AOs can be designed to target an RNA molecule with exquisite specificity and have delivered benefit to patients living with previously untreatable rare diseases. AO therapeutics in development for treating neurological diseases use the phosphorothioate chemistry that has demonstrated inherent toxicity, including activation of innate immunity, plasma protein binding, and nuclear aggregation of RNA-binding proteins. Here, we investigate the utility of a different chemical class of neutral AOs called phosphorodiamidate morpholino oligomers (PMOs) for treating CNS disorders. PMOs have proven safe and effective in the clinic when delivered systemically to treat Duchenne muscular dystrophy; we hypothesise that high doses of PMO delivered into the CNS will be safe and effective. Here, we investigate the preliminary biodistribution and safety of high doses (up to 2.5 µg) of a PMO administered via an intracisternal magna injection in the CNS of wild-type mice. After 7 days, brains and spinal cords were collected from PMO-treated and vehicle control mice and snap frozen in isopentane. To determine the regional distribution of the PMO in the CNS, we performed miRNAscope™ (ACDBio), with probes that are complementary in sequence to the PMO, on serial sagittal brain sections and spinal cord sections. In addition, we performed a preliminary safety assessment using a blood biochemistry panel. MiRNAscope™ showed the PMO distributed across the CNS. No significant changes in blood biochemistry or liver and kidney histopathology were detected. We showed that high doses of a PMO in the CNS was safe and distributed to mouse CNS tissues and motor neurons, indicating that unconjugated PMOs at high doses may be an option for AO therapeutics treating neurological diseases.

From Patient to Platform: Establishing Orthotopic AT/RT and Pineoblastoma PDX Models with Tailored Irradiation Protocols

Hilary Hii[1], Meegan Howlett[1,2], Jacqueline Whitehouse[1,2], Hetal Dholaria[1,3,4], Jessica Buck[1,2], Annabel Short[1,2], Amanda Luck[5], Jacob Byrne[1], Brooke Carline[1], Piyusha Johnson[1], Chelsea Mayoh[6,7], Marie Wong[6,7], Jordan Hansford[5], Nick Gottardo[1,3], and Raelene Endersby[1,2]

[1]The Kids Research Institute of Australia, [2]Centre for Child Health Research, UWA, [3]Department of Paediatric Oncology/Haematology, Perth Children's Hospital, [4]Division of Paediatrics, UWA Medical School, [5]The Women's and Children's Hospital; South Australia Health and Medical Research Institute, [6]Children's Cancer Institute, [7]School of Women's and Children's Health, Lowy Cancer Centre, UNSW

Introduction. Atypical teratoid/rhabdoid tumours (AT/RT) and pineoblastoma (PB) are rare, aggressive embryonal brain tumours that primarily affect infants under 3 years old. Prognosis remains abysmal, with a median survival of just 36 months. **Problem Statement.** Due to their rarity, there are no standardised treatment protocols or active clinical trials for these patients. Preclinical testing has also been limited by the absence of disease-specific models. Radiotherapy is often avoided in this age group, further limiting treatment options. However, some evidence suggests that focal irradiation may improve survival. We aim to address this underserved population by developing patient-derived orthotopic xenograft (PDOX) models, then optimising brain irradiation protocols for the models as a foundation for future preclinical testing. **Procedures/Data/Observations**. PDOXs TK-ATRT953, TK-ATRT992, TK-PB987 and TK-PB453 were developed in-house and tumours compared to the corresponding primary specimens via STR analysis, DNA methylation profiling, whole genome and RNA sequencing. Whole brain irradiation was delivered as 10 x 1.0 Gy or 20 x 1.0 Gy fractions using a 5-days-on, 2-days-off schedule. **Results**. STR profiles, along with DNA methylation profiling, whole genome sequencing, and RNA sequencing data from all PDOXs closely match those of the primary patient tumour. Optimal whole brain irradiation schedules of 10 Gy for TK-PB987 and 20 Gy for TK-PB453 PDOX models were determined, which resulted in significantly increased survival compared to non-irradiated controls ($p<0.0001$), but did not cure mice of tumours. This reflects clinical outcomes and allows scope for the assessment of novel, radio-sensitising compounds in future preclinical studies. **Conclusions**. We have established multiple PDOX models of these rare and aggressive embryonal brain tumours and developed clinically relevant irradiation protocols for future preclinical testing. We aim to utilise these valuable tools to identify effective new treatment options for these patients that currently have an almost universally fatal outcome.

Antisense oligonucleotide mediated reduction of C9ORF72 expansion containing transcripts in iPSC-derived motor neurons

Leon M. Larcher[1], Ianthe Pitout[1], Rakesh N. Veedu[1, 2], Sue Fletcher[1]

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

Introduction. Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting motor neurons. A G_4C_2 repeat expansion in intron 1 of the *C9ORF72* gene is the most common genetic cause of ALS and induces neurodegeneration by; (1) C9ORF72 loss-of-function; (2) RNA toxic gain-of-function; and (3) Gain-of-function through accumulation of toxic dipeptide repeat peptides (1). Removal of the G_4C_2 expansion in *C9ORF72* partially ameliorates the gain-of-function mechanisms (2). **Problem Statement**. Despite the recent failures of RNase H-dependent oligonucleotide drugs in C9ORF72-linked ALS clinical trials, therapeutic strategies to suppress expression of the toxic *C9ORF72* variants containing the G_4C_2 expansion repeat still have enormous disease-modifying potential for ALS. **Procedures/Data/Observations** In this project, we employed a steric-blocking antisense oligonucleotide strategy to mediate the reduction of expansion-containing *C9ORF72* transcripts in C*9ORF72* ALS patient dermal fibroblasts and iPSC-derived motor neurons. Splice-switching phosphorodiamidate morpholino oligomers (PMOs) targeting *C9ORF72* were electroporated into patient iPSC-derived motor neurons. **Results** Analysis of *C9ORF72* transcript variant 3 (expansion containing), variant 2 (alternative transcript lacking the expansion), and all transcript variants were assayed by droplet digital PCR. RNA foci containing the *C9ORF72* G_4C_2 expansion were measured *in situ* using complementary probes and a Basescope^{TM} assay. Gene expression analysis revealed that our PMOs could selectively reduce levels of variant 3 by >90% compared with the sham control and did not reduce levels of variant 2 or all transcript variants. Additionally, reduction of variant 3 also reduced the formation of G_4C_2 expansion containing RNA foci. **Conclusions**. Here, we show evidence for PMO-induced suppression of the toxic *C9ORF72* variant 3 *in vitro* using C9ORF72-linked ALS patient iPSC-derived motor neurons, suggesting that utilising an isoform switching mechanism of action and PMO chemistry may have therapeutic potential for C9ORF72-linked ALS, offering another opportunity for these patients.

Comparative Analysis of Strep A Bacterial Detection Methods in an Australian Paediatric Urban Pharyngitis Surveillance Study

A. Takahashi[1,2,3], J. Voss[1,4], S. Yeow[1,4], M.V. Morici[1,2,3], H.R. Frost[1,4], A.M. Messiano[1,2,3], C. Tedja[1,2,3], A.J. Hayes[5], Z.Y. Ng[1,2,3], E.J. Negus[1,2,3], A.C. D'Aprile[1,2,3], J. Pickering[1,2,3,6], C.A. Baker[1,4], M. Angel, T. Mills, M.E. Duenas[1,2,3,6], T. Williams[1,4], M.R. Davies[5], A.C. Steer[1,4], J.R. Carapetis[1,2,3,6,7], A. Fulurija[1,2,3,6]

[1]The Australian Strep A Vaccine Initiative (ASAVI), [2]Wesfarmers Centre for Vaccines and Infectious Diseases, [3]The Kids Research Institute Australia, [4]Murdoch Children's Research Institute, [5]The University of Melbourne, [6]The University of Western Australia, [7]Perth Children's Hospital

Streptococcus pyogenes (Strep A) causes around 500,000 deaths annually, disproportionately affecting developing countries and Indigenous populations, including Aboriginal Australians. The Australian Strep A Vaccine Initiative aims to accelerate Strep A vaccine development by generating surveillance data on pharyngitis rates, Strep A carriage, and *emm* types, which are critical for assessing strain coverage among Australian children and informing vaccine trials. Despite significant research, surveillance data on paediatric non-invasive Strep A infections in Australia remains limited. Comparative data on the performance of diagnostic methods are also limited, hindering efforts to identify optimal approaches for bacterial detection. The Sore Throat Melbourne and Perth Study (STAMPS) collected throat swabs from children aged 3–14 years at enrolment (baseline), seasonal follow-ups, and symptomatic sore throat trigger visits (STTVs). Swabs were tested using three methods: Abbott ID NOW^{TM} *Strep A2* point-of-care nucleic acid amplification test (POC-NAAT), reference standard bacterial culture (RSBC) and broth-enriched bacterial culture (EB). Isolates underwent whole genome sequencing. Diagnostic performance was evaluated by comparing detection rates and assessing method agreement. Among 525 throat swabs collected from healthy children in Perth (baseline and seasonal), 55 were culture-positive by RSBC, EB or both, with 65.5% overlap in detection between the two culture methods. Detection rates between RSBC and EB were not statistically different (McNemar's test, p= 0.21), with strong agreement between methods (Cohen's kappa = 0.80). Predominant *emm* types were 12.0, 1UK, 28.0, and 89.0.Of 376 STTVs where POC-NAAT was applied, 127 were positive by POC-NAAT, RSBC or both. POC-NAAT identified significantly more positives than RSBC (McNemar's test, p<0.0001), with agreement between methods as expected (Cohen's kappa = 0.77). The most frequently detected *emm* types were 12.0, 1UK and 89.0.EB and RSBC showed comparable performance, while POC-NAAT demonstrated superior sensitivity. Culture-based methodology is limited when detecting low bacterial loads or non-viable Strep A.

Pharmacological inhibition of sclerostin protects bone from B-cell acute lymphoblastic leukemia-mediated destruction

Vincent Kuek[1,2,3], Joyce Oommen[1], Emanuela Ferrari[1], Rishi S. Kotecha[1,2,3], Laurence C. Cheung[1,2]

[1]The Kids Research Institute Australia, [2]Curtin Medical School and Curtin Medical Research Institute, Curtin University, [3]School of Biomedical Sciences and UWA Medical School, University of Western Australia

Introduction. Around 26% of children with acute lymphoblastic leukaemia (ALL) develop incidence of vertebral fractures in the 4 years post-diagnosis. Without intervention, these children are predisposed to increased risk of osteoporosis and bone fractures, which may pose significant personal and health burdens. Therefore, novel approaches to prevent and/or treat leukaemia-induced bone loss are desperately needed. **Problem Statement**. We evaluated the therapeutic strategy of pharmacologically restoring osteoblastic cells (OBCs) in murine B-ALL models using a neutralising antibody targeting sclerostin (Scl-Ab), a protein which inhibits bone formation. **Procedures/Data/Observations**. We evaluated the efficacy of Scl-Ab treatment in the bones of two murine B-ALL models with severe bone loss: an immunocompromised patient-derived xenograft (PDX) NSG model of relapsed B-ALL (ALL-84) and an immunocompetent BCR-ABL1^{+} syngeneic model of B-ALL (PER-M60). We also evaluated the impact of Scl-Ab treatment on the survival of these leukaemia-bearing mice. **Results**. We found that Scl-Ab treatment significantly increased the number of femoral OBCs in both the ALL-84 leukaemia-bearing mice and PER-M60 leukaemia-bearing mice when compared to their respective control leukaemia groups treated with isotype IgG antibody (Iso-Ab). Furthermore, micro-CT analyses revealed that Scl-Ab treatment improved bone parameters in both leukaemia models when compared to their control Iso-Ab treatment groups. Histological analyses revealed that Scl-Ab treatment did not affect the osteoclast parameters, suggesting the specificity of Scl-Ab in targeting the OBCs to promote bone formation and bone health. Remarkably, PER-M60 leukemia-bearing mice treated with Scl-Ab demonstrated a modest but significant extension of survival compared to Iso-Ab treated mice. **Conclusions**. Our preclinical study has shown that targeting OBCs is a promising therapeutic strategy to restore ALL-induced bone loss. Future clinical studies should investigate the incorporation of Scl-Ab into conventional treatments for children with high-risk B-ALL who are at increased risk of developing incident fractures.

Copper That! Improving immunotherapy response rates in mesothelioma by targeting copper

Naomi Damstra[1,2], Joshua Ravensdale[2], Nicola Principe[2], Amber-Lee Phung[2], Andrew Crowe[1,3], Ross Graham[1], Orazio Vittorio[4], Delia Nelson[1,3], Jonathan Chee[2], Kofi Stevens[1,2,3]

[1]Curtin Medical School, Curtin University, [2]Centre for Respiratory Health, National Centre for Asbestos Related Diseases, University of Western Australia, [3]Curtin Health Innovation Research Institute, Curtin University, [4]Children's Cancer Institute, University of New South Wales

Australia has one of the highest incidence rates of the asbestos related cancer, mesothelioma. The expected survival time is around 12 months with standard doublet chemotherapy. The introduction of immune checkpoint inhibitors (ICIs) has improved the average survival time by up to 18 months, a substantial improvement from dual chemotherapy. However, only 20-30% of treated patients benefit from ICI, thus our work aims to utilise copper binding drugs as a novel way to increase the number of responders. Copper (Cu) is essential for healthy cells. However, Cu has been found to accumulate in many cancers including mesothelioma, due to its roles in energy production, free radical detoxification, angiogenesis, and mesenchymal transition. Using the clinically approved Cu-chelator Triethylenetetramine (TETA), we aim to reduce Cu available to the tumour, and understand how it improves the function of anti-cancer immune cells in mesothelioma. To accomplish this, we have characterised the tumour microenvironment (TME) of untreated and Cu-chelation treated murine mesothelioma tumours using a 20+ colour spectral flow cytometry panel, while spatially mapping key immune cells and angiogenesis markers via multiplex immunofluorescence. Further, we have determined the abundance and localisation of Cu with subcellular resolution within the TME using X-Ray fluorescence microscopy at the Australian Synchrotron. The data from these experiments has been used to optimise combination Cu-chelation therapy and α-PD-1/α-CTLA4 immunotherapy. We have found that combination treatment increases overall survival and significantly reduces tumour growth. Furthermore, we see that Cu-chelation therapy alters the tumour infiltrating lymphocytes (TILs), likely contributing to the observed increased response rates to ICI treatment. Our preliminary data is the first to demonstrate the therapeutic effect of Cu chelators as an effective treatment for mesothelioma. Data from our *in vivo* experiments will provide the scientific rationale for developing novel Cu-based strategies to improve clinical responses to immunotherapy in mesothelioma and other cancers. Cu-chelators are already clinically approved for the life-long treatment of Wilsons disease (a Cu accumulation disease) and therefore could be easily repurposed for the treatment of cancer.

Evaluation Of A Total IgG Normalization Of Antigen Specific IgG Data From Variably Collected Capillary Blood Samples

Chantalia Tedja[1,2,3], Anna Takahashi[1,2,3], Michael Morici[1,2,3], Hannah Frost[1,4], Christian Tjiam[2,3,6], Zi Ying Ng[1,2,3], Serene Yeow[1,4], Andrew Steer[1,4], Jonathan Carapetis[1,2,3,5,7], Alma Fulurija[1,2,3,5]

[1]The Australian Strep A Vaccine Initiative (ASAVI), [2]Wesfarmers Centre for Vaccines and Infectious Diseases, [3]The Kids Research Institute Australia, [4]Murdoch Children's Research Institute, [5]The University of Western Australia, [6]PathWest Laboratory Medicine WA, [7]Perth Children's Hospital

The Sore Throat Melbourne and Perth Study (STAMPS), led by The Australian Strep A Vaccine Initiative, aims to determine the prevalence and incidence of *S. pyogenes* (Strep A) pharyngitis, including serologically-confirmed cases in Australian children aged 3-14 years. Capillary blood samples were collected using Mitra devices based on volumetric absorptive micro-sampling (VAMS) technology-a less invasive alternative to venipuncture that maintains sample integrity for accurate serological analysis. This study provides the first comprehensive data on serologically confirmed Strep A pharyngitis in Australian children, enhancing our understanding of antibody responses to Strep A antigens and informing future vaccine trials. Variable saturation of VAMS tips during blood collection creates challenges for accurate quantification of antigen-specific antibodies, as inconsistent sample volumes result in variable dilution factors during elution. To overcome this limitation, we developed a normalization method using a total IgG ELISA to standardize antigen-specific IgG concentrations measured by a multiplex MSD assay, enabling accurate antibody comparison across samples regardless of Mitra tip saturation status. Controlled whole blood volumes (2.5-30 µL) were prepared and analyzed by total IgG ELISA to validate the normalization strategy. VAMS tips were visually assessed and categorized by saturation percentage relative to full capacity. Visual estimates correlated closely with measured IgG levels, with tips estimated at 80% saturation yielding 83.6% of the total IgG concentration observed in fully saturated tips. A strong linear correlation was observed between measured blood volume and average calculated IgG concentration (r= 0.995; 95% CI: 0.995-0.999). Visual saturation estimates also showed a strong correlation with average IgG Concentration (r= 0.963; 95% CI: 0.769-0.995). The strong correlation between sample blood volume and observed IgG concentration validates the total IgG ELISA as a normalization method for partially filled VAMS tips. This approach effectively compensates for variable sample volumes ensuring that antibody measurements remain accurate and comparable.

A Novel Pipeline for Innovative Peptide-Based Diagnostics

Samuel Lundin[1], Luke Marshall[1], Paige Warburton[1], Soraya Leedham[1], Paige Bebee[1] and Alma Fulurija[1]

[1]Biotome Pty Ltd

Unlike protein-based serology, peptide-based diagnostics are of high-specificity, low cost and minimal cross-reactivity. To create effective peptide-based serological assays a pipeline was developed and applied to the creation of two prototypes: a test for CagA *H. pylori* strains and an assay for preeclampsia risk.

Four phases of development were used:Immune Response and Target Protein Selection: Immune responses and selected target proteins were analysed from the existing literature and known antigenic proteins. This aimed to identify likely candidates for diagnostic purposes, focusing on their relevance in disease pathogenesis. Linear Epitope Mapping: High-density peptide arrays were employed to perform epitope mapping and screen thousands of targets for potentially diagnostic peptides. This process allowed for the identification of peptides that elicited strong and specific antibody responses, suitable for diagnostic use. Epitope Refinement and Determination of Diagnostic Peptides: Following the initial mapping, peptide microarrays were used to refine the identified epitopes and determine the most diagnostic peptides, resulting in the identification of epitopes with the highest specificity for the target condition. Prototype Peptide ELISA Development and Validation: A prototype peptide-based ELISA was then developed and validated in larger cohorts. Optimisation was conducted to maximise assay performance, ensuring reliability and reproducibility. Through the application of this pipeline a peptide-based serology assay was developed allowing for the accurate detection of *H. pylori* CagA antibodies. In a pilot study (n=32) the assay accurately discriminated between CagA-positive and CagA-negative *H. pylori* infections. This pipeline was also applied to a Swedish preeclampsia case-control cohort (n=31), identifying that a subset of preeclampsia cases exhibited elevated anti-CagA IgG responses. Our research demonstrates the value of peptide-based diagnostics for developing specific serological assays. The robust, systematic epitope mapping approach offers an efficient research pipeline for immunoassay diagnostics for infectious disease and autoimmunity targets.

Autoantibodies as potential biomarkers of response to immune-checkpoint inhibitor (ICI) immunotherapy in cutaneous melanoma patients

Pauline Zaenker[1,2], Aesha Gandhi[1,2,3,8], Desiree Sexauer[1,2], Anna Reid[1,2], Tarek Meniawy[4,5], Muhammad Adnan Khattak[6,7], Michael Millward[8], Elin Gray[1,2]

[1]Centre for Precision Health, Edith Cowan University, [2]School of Medical and Health Sciences, Edith Cowan University, [3]Royal Perth Hospital, [4]St John of God Hospital, [5]Sir Charles Gairdner Hospital, [6]Fiona Stanley Hospital, [7]Hollywood Private Hospital, [8]The University of Western Australia

Introduction: ICI immunotherapy has revolutionised the melanoma treatment landscape. However, approximately half of all patients do not respond to ICI and predictive biomarkers are urgently needed. We evaluated IgG autoantibody (AAb) levels in patient samples to identify AAbs that predict treatment response. **Methods:** Baseline serum samples were collected from 85 metastatic cutaneous melanoma patients treated with pembrolizumab or nivolumab monotherapy (n=48 'PD1 cohort') or combination ipilimumab and nivolumab (n=37 'Ipi/Nivo cohort'). Response to treatment was evaluated using the iRECIST guidelines and assessed via routine PET/CT scan 12 weeks after therapy commencement. To profile serum antibodies, HuProt™ Microarrays v4 (CDI Labs) were scanned on an Innoscan 710AL (Innopsys) microarray scanner with Mapix software. The significance of AAb level differences between treatment responders and non-responders was assessed by Mann-Whitney-U comparison. A serum score was calculated to quantify patient seroreactivity for multiple antibodies and ROC analysis determined biomarker sensitivity and specificity. The association between AAb levels and survival was examined with Kaplan-Meier survival curves. **Results:** Samples were screened for IgG antibodies targeting a total of 23059 proteins. 77 and 66 antibodies were identified with significantly elevated levels in the ICI responder baseline samples (PD1 cohort and Ipi/Nivo cohort, respectively). All identified antibodies displayed 2 - 5.9-fold differences in levels between responders and non-responders. The maximum AUC for a single antibody of interest was 0.848. Patients with seroreactivity for multiple identified AAbs at baseline (serum score >1000) had significantly better overall and progression-free survival than patients with less seroreactivity at baseline ($p<0.0001$). **Conclusions:** Autoantibodies may serve as useful biomarkers for the prediction of ICI response at baseline. A total of 50 antibodies of interest have now been selected for validation using focus protein microarrays. Further testing is currently underway to add to these preliminary findings and commence the necessary validation of the identified biomarkers.

Development of 10-Plex Electrochemiluminescent Assays for Quantifying Specific Serological Immunoglobulin Isotype and Subclass Responses to Streptococcus pyogenes Antigens

Michael Morici[1,2,3], Jill Gilmour[1,4], Hannah Frost[1,5], Joshua Osowicki[1,5], Andrew Steer[1,5], Jonathan Carapetis[1,2,3,6,7], Alma Fulurija[1,2,3,6]

[1]The Australian Strep A Vaccine Initiative, [2]The Kids Research Institute Australia, [3]Wesfarmers Centre for Vaccines and Infectious Diseases, [4]JWG Global Lab Solutions, [5]Murdoch Children's Research Institute, [6]University of Western Australia, [7]Perth Children's Hospital

The global burden of *Streptococcus pyogenes* is a major unmet public health challenge. A safe and effective vaccine could substantially reduce the impact of this pathogen, particularly in low-resource settings where the disease burden is highest. To support vaccine development, standardized immune assays are required to generate reproducible and comparable antigen-specific serological data. Future vaccine trials, particularly those involving young children, will require assays that are highly scalable, reproducible, and precise, while also using only small sample volumes. To meet these needs, we developed 10-plex electrochemiluminescence-based assays using the Meso Scale Discovery (MSD) platform to quantify IgG, IgG subclasses, and IgA recognizing the *S. pyogenes* antigens streptolysin O, Deoxyribose nuclease B, *S. pyogenes* cell envelope protease, *S. pyogenes* adhesion and division protein, Streptococcal C5a peptidase, Group A Carbohydrate, Nuclease A, Spy0843/Leucine-rich repeat domain-containing protein, Oligopeptidepermease, and pullulanase. Assay parameters were optimized and performance evaluated using healthy donor samples. Specificity, dilutional linearity, and limits of quantitation were assessed. The assays demonstrated excellent precision, with inter-assay CVs <20% for all protein antigens, high sensitivity, and a broad dynamic range for IgG (1.71-3.66 log), as well as for IgG subclasses, and IgA. Standard curves were generated using IVIG for IgG and IgG subclasses and pooled serum for IgA, enabling calculation of concentrations in arbitrary units/mL (AU/mL) and interpolation of sample concentrations. The 10-plex assays provide a robust, flexible platform that builds on the previously reported 6-plex format and adds detection options for IgA and IgG subclasses, making them ideal for seroprevalence studies, natural infection and human challenge studies, and advancing understanding of humoral immune responses to *S. pyogenes* antigens. By enabling precise and scalable measurement of antigen-specific antibody responses, these assays offer a key tool to support more nuanced immunogenicity assessments in future *S. pyogenes* vaccine trials.

Loop of unknown origin – Discovery of a novel 24 kb circular element harbouring viral-like genes in global populations of Ascochyta pathogens of lentil and chickpea

Johannes Debler, Robert Lee, Bernadette Henares, Lars Kamphuis, Bethany Clark, Hediyeh Tahghighi, Karam Singh

Centre for Crop and Disease Management, Curtin University

Ascochyta blight (AB), caused by the necrotrophic and hemibiotrophic fungal pathogens Ascochyta rabiei and Ascochyta lentis, represents the most economically damaging fungal disease of chickpea and lentil crops worldwide, respectively. Through comprehensive long-read genome sequencing of isolates from both pathogen species collected across multiple continents and spanning over three decades, we identified a novel circular genetic element encoding virus-like genes present in select isolates from geographically distant regions. Phylogenetic analysis revealed that some variants of this element show greater sequence similarity between the two distinct Ascochyta species than among isolates within the same species, providing compelling evidence for horizontal gene transfer. This finding is particularly intriguing given the strict host specificity typically exhibited by these pathogens, with A. rabiei exclusively infecting chickpea and A. lentis restricted to lentil hosts. The discovery of this mobile element suggests previously unrecognized mechanisms of genetic exchange between these economically important plant pathogens and raises questions about the evolutionary forces shaping pathogen populations across legume crops. Our results highlight the power of long-read sequencing technologies in revealing cryptic genomic features that may influence pathogen evolution and disease dynamics in agricultural systems.

Start Right to End Right: Authentic Open Reading Frame Selection Matters

Mojtaba Bagherian[1], Georgina Harris[1], Pratosh Sathishkumar[1], and James P B Lloyd[1,2]

[1]ARC Centre of Excellence in Plant Energy Biology, School of Molecular Sciences, The University of Western Australia, Perth, Australia; [2]ARC Centre of Excellence in Plants for Space, School of Molecular Sciences, The University of Western Australia, Perth, Australia

Introduction. Accurate identification of start codons is critical for proper annotation of open reading frames (ORFs) and prediction of nonsense-mediated mRNA decay (NMD) targets, a conserved eukaryotic quality control pathway that degrades transcripts with premature termination codons. **Problem Statement**. Current genome annotation algorithms frequently select the longest ORF rather than the authentic start codon, leading to mischaracterization of NMD features and contamination of protein databases with computational artifacts. **Procedures**. We applied TranSuite software to re-annotate the *Arabidopsis thaliana* Araport11 transcriptome, comparing reference ORF annotations with revised authentic start codon predictions. RNA-seq data from wild-type and an NMD-deficient double mutant (*upf1-1 upf3-1*) were analyzed to identify putative NMD targets based on increased steady-state expression. We assessed the frequency of NMD-inducing features, specifically premature termination codons with downstream exon junctions (PTCdEJ). AlphaFold3 was used to demonstrate structural differences between incorrect and correct ORF predictions. **Results**. Reference ORF annotations identified only 203 upregulated transcripts with PTCdEJ features, while revised ORF annotations revealed 426 such transcripts, more than doubling NMD target identification. The NMD enrichment factor increased from 2.4 to 2.9, demonstrating superior discrimination between true NMD targets and indirect transcriptome changes. Analysis of 3' UTR lengths showed significant differences between annotation methods. Protein structure predictions revealed dramatic differences between the longest ORF and authentic ORF annotations. **Conclusions**. Authentic start codon selection dramatically improves NMD target prediction and prevents protein database contamination with computational artifacts. Our findings demonstrate that the longest ORF selection ignores biological reality, as ribosomes select start codons independently of downstream alternative splicing events, with critical implications for all eukaryotic genome annotation projects.

A superhelical filament forming oligomeric wing-helix-turn-helix domain DNA-binding protein controls horizontal transfer of symbiosis genes

Callum J. Verdonk[1 2 3], Mark Agostino[2], Karina Yui Eto[1 2], Drew A. Hall[2 4 5], Charles S. Bond[1 6] and Joshua P. Ramsay[2 4]

[1]School of Molecular Sciences, The University of Western Australia; [2]Curtin Medical Research Institute, [3]Centre for Crop and Disease Management & [4]Curtin Medical School, Curtin University, [5]Australian National Phenome Centre, Murdoch University, [6]Marshall Centre for Infectious Disease Research and Training, The University of Western Australia

RdfS is a critical DNA-binding protein controlling the chromosomal excision and horizontal gene transfer of genes for nitrogen-fixing symbiosis in members of the genus *Mesorhizobium*. Here we present the X-ray crystal structure of RdfS and reveal it forms superhelical filaments in head-to-tail orientation and a continuous positively charged surface likely contacting DNA. RdfS is a member of the MerR family of transcriptional regulators and contains a winged helix-turn-helix (wHTH) DNA-binding domain with a highly disordered C-terminal tail. We show RdfS binds to DNA regions within the IntS attachment site (*attP*) and within the *rdfS* promoter, enabling RdfS to coordinate *rdfS*/*intS* expression and stimulate RdfS/IntS-mediated ICE*Ml*Sym^{R7A} excision. Several RdfS DNA-binding sites were identified. However, no consensus motif was apparent and no individual nucleotide substitutions in *attP* prevented RdfS binding. RdfS forms extensive helical filaments in crystals, with subunits contacting via a novel α1-helix absent in other wHTH-RDFs. RdfS oligomerised in solution in the absence of DNA. Molecular dynamics simulations supported a role for the α1-helix in oligomerisation and compaction of nucleoprotein complexes. Removal of RdfS-α1 did not eliminate DNA-binding *in vitro* but reduced oligomerisation and abolished RdfS-mediated ICE*Ml*Sym^{R7A} excision and conjugative transfer. We propose the novel RdfS-α1 mediated oligomerisation enables RdfS to specifically recognise larger DNA regions with low primary sequence conservation through an indirect read-out mechanism.

Panning for Gold in Mould: How to increase the odds for genome mining of bioactive secondary metabolites in fungi?

Heng CHOOI
School of Molecular Sciences, The University of Western Australia, Perth WA 6009, Australia

Fungal secondary metabolites are a double-edged sword: they underpin transformative medicines and agrochemicals, yet also include notorious mycotoxins and virulence factors of human and plant pathogens. In the genomics era, a clear paradigm has emerged — microbial genomes harbour far more biosynthetic gene clusters (BGCs) than the chemical diversity revealed under standard laboratory cultivation. Heterologous pathway reconstruction has therefore become a powerful route to translate cryptic BGCs into bioactive molecules. But how do we increase the odds of success when mining the vast fungal genomic landscape?

I will present several strategies we deploy, including (i) host–pathogen interaction transcriptomics to uncover cryptic virulence-associated metabolites; (ii) taxonomy-guided discovery targeting endemic Australian fungi; and (iii) resistance-gene-guided genome mining. The latter led to elucidation of the biosynthetic pathway to cerulenin, the first reported natural fatty-acid synthase inhibitor, discovered over six decades ago. We show that cerulenin biosynthesis begins with a C12 precursor produced by a polyketide synthase, rather than via fatty-acid biosynthesis. This precursor contains both E and Z double bonds and undergoes amidation followed by a sequence of epoxidations, double-bond migrations, E/Z isomerisation, and epoxide reduction. These findings illustrate how informed genome-mining strategies can accelerate discovery of bioactive fungal metabolites and deepen connections between BGC sequences, metabolite structures, and modes of action.

Transposon-Associated Adaptation of The Australian *Parastagonospora nodorum* Population

Huyen T. T. Phan[1†]*, Manisha Shankar[2†], Darcy A. B. Jones[1], Eiko Furuki[1], Kasia Rybak[1], Fiona Kamphuis[1], Hossein Golzar[2], Richard P. Oliver[3]

[1]Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Perth, WA, [2]Department of Primary Industries and Regional Development, [3]University of Nottingham, Nottingham

Septoria nodorum blotch (SNB) is an economically important fungal disease of wheat caused by *Parastagonospora nodorum*. It is common in Australia, USA and Norway. In Western Australia alone, losses due to the disease was estimated AU$112m p.a., second amongst all diseases of wheat (*Triticum aestivum*). To effectively control the disease, knowledge about the pathogen's evolutionary potential is pivotal. This information will help to understand how this pathogen can adapt and overcome resistance cultivars. In this study, we assembled a panel of 360 *P. nodorum* isolates from across the West-Australian wheatbelt, dating from 1968 to 2021, and determined the population structure using 18,208 biallelic SNP datapoints covering the entire pangenome. Result from population diversity analysis identified a structure of 8 groups. One core population was found from many collection locations and across the entire period. Seven other transient populations were found in restricted locations and times. The core population had a balanced ratio of mating types 1 and 2, divergent necrotrophic effector haplotypes and a variable content of intact and degraded copies of a Tc-1 mariner transposon, called Molly. In contrast, the transient groups were almost entirely monomorphic at the mating type and necrotrophic effector loci and their Molly content. When tested on seedlings, the recently emerged transient groups exhibited greater pathogenicity on modern elite wheat cultivars consistent with the low-amplitude boom-and-bust cycle observed previously in this pathosystem. The data are consistent with a model whereby strains adapted to contemporary dominant wheat cultivars emerged from the core population and expanded primarily by asexual reproduction. It is possible that active copies of Molly transpose and contribute to both the birth and death of the transient groups. The study suggests practical measures that can be undertaken to improve the efficiency and longevity of resistance breeding for SNB.

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Development of strategies and genomic resources to minimise the impact of Australian wheat powdery mildew

Cao (Grace) Fang [1], Julie Lawrence [1], Ancy Tony [1], Fran Lopez [1], Simon Ellwood [1], Kar-Chun Tan [1]

[1] The Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Bentley, Australia

Introduction. Wheat powdery mildew (WPM) is caused by the obligate biotrophic fungus *Blumeria graminis*. WPM causes significant yield and economic losses to the Australian cereal industry. Current strategies to minimise the impact of WPM include crop rotation, chemical control and genetic resistance. **Problem Statement**. The continuous use of fungicides to control WPM has resulted in the emergence of fungicide-resistant *B. graminis* isolates across Australia. This highlights the need to identify an alternative strategy to minimise the impact of WPM on the Australian cereal industry. Despite its economic importance, there is little knowledge on effective host resistance in Australian wheats and local genetic diversity/population structure. **Procedures**. Our primary goal is to work with the Australian cereal industry to develop durable management strategies that adopt broad-spectrum WPM resistance in wheat or to identify major resistance (*R*) genes from diverse wheat germplasm collections. Once validated to be effective against different *B. graminis* pathotypes, we will introduce and stack *R* genes into breeding stocks. Furthermore, we will establish extensive genomic resources for Australian *B. graminis* isolates with the goal of understanding its population structure, mechanism of fungicide resistance, genomic diversity and the identification of avirulence effectors, where they can then be exploited as a molecular tool for novel *R* gene discovery.

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People, Parks and PCRs: interweaving of environmental DNA (eDNA) to enhance Ocean conservation

Eric J. Raes[1,2], Marcelle E. Ayad[1,2], Philipp E. Bayer[1,2], Shannon Corrigan[1,2], Matthew Fraser[1,2], Priscila Goncalves[1,2], John de Majnik[3], Alex Tomlinson[3] & OceanOmics Centre[2], Michael Bunce[1,2]
1. Minderoo Foundation, Broadway, Nedlands, Australia 2. Minderoo OceanOmics Centre at UWA, Oceans Institute, The University of Western Australia, Crawley, Australia 3. DCCEEW, Parks Australia

Marine ecosystems are increasingly threatened by climate change, invasive species, biodiversity loss, overfishing, and pollution. Protecting Australia's oceans and marine parks requires a collective approach - one that includes Indigenous, community, scientific, and industry-led efforts. Environmental DNA (eDNA) is emerging as a powerful tool to enhance our understanding of ocean biodiversity and ecosystem health. Despite its potential, the integration of eDNA into national marine monitoring remains limited. Traditional methods dominate, and the lack of accessible, user-friendly tools hinders the broader application of eDNA for routine monitoring and conservation planning. In partnership with Parks Australia, the Minderoo Foundation has processed over 6,000 eDNA samples from diverse locations spanning the Indian Ocean Territories to Tasmania. Here, we will showcase how novel AI-based approaches such as data chatbots, taxonomic classifiers, and clustering algorithms can be used to extract, curate and translate eDNA information. These innovations coupled with visualisation dashboards enable end users to engage with eDNA data, explore species distributions, track biodiversity and ecosystem health trends over time. The integration of eDNA with AI and visualization tools will demonstrate the ability to efficiently process thousands of samples, identify taxa across trophic levels, and reveal spatial biodiversity patterns. These tools support the development of a 'triage' layer for marine monitoring- enabling rapid biodiversity assessments that can inform deeper investigations when necessary. Our work contributes to national efforts to modernize biodiversity monitoring and foster inclusive ocean stewardship. Ongoing Minderoo partnerships - including with the IUCN Red List, CSIRO (eDNA autosamplers), and community programs with UNESCO - are accelerating the advancement of eDNA as a central tool for sustained marine conservation.

Embedding environmental DNA in the IUCN Red List of Threatened Species

Matthew W. Fraser[1,2], Philipp E. Bayer [1,2], Marcelle E. Ayad[1,2], Shannon Corrigan[1,2], Eric J. Raes[1,2], , Priscila Goncalves[1,2] & OceanOmics Centre[2], Michael Bunce[1,2], Neil Cox[3]

[1]Minderoo Foundation, Broadway, Nedlands, Australia. [2]Minderoo OceanOmics Centre at UWA, Oceans Institute. [3]International Union for the Conservation of Nature, Washington D.C., U.S.A.

The IUCN Red List of Threatened Species is widely used globally to guide decision-making around species conservation priorities. The assessment process is driven by a group of dedicated volunteers. Given the importance of the Red List for conservation decision-making there is a critical need for species to be assessed frequently and comprehensively, with new data guiding assessment processes when available. Environmental DNA (eDNA) is an emerging technique for monitoring species in space and time, but eDNA is currently not typically being used in the Red List Assessment process. This project aimed to develop guidelines and tools for Red List Assessors to begin to incorporate eDNA-derived data into species extinction risk assessments. We piloted this approach at a Red List Assessment workshop in August 2025 that assessed the extinction risk of 680 fish species around Australia and New Zealand. We provide broad recommendations around how we should aim to incorporate eDNA data for the Red List of Threatened Species, and how this can be integrated along with other emerging technologies to help make the Red List more responsive to real-world biodiversity trends.

Laboratory automation for high-throughput metabarcoding and amplicon sequencing of thousands environmental DNA samples

Dang Sang Huynh[1], Laura Missen[1], Marcelle Ayad[1,2], Liam Anstis[1], Adrianne Doran[1], John Blinco[1,3], OceanOmics Division[1,2], OceanOmics Centre[1], Shannon Corrigan[1,2]

[1]Minderoo OceanOmics Centre at UWA, Oceans Institute, University of Western Australia, [2]Minderoo Foundation, Perth, WA, Australia. [3]Current position in RNA Innovation Foundry, School of Human and Molecular Sciences, University of Western Australia

Environmental DNA (eDNA) technology has become a powerful tool for assessing and monitoring biodiversity across diverse ecosystems. At Minderoo OceanOmics Centre at University of Western Australia, where we specialise in eDNA-based approaches, the volume of samples collected during research voyages or received from collaborators is substantial. To meet the growing demand, we recognised the need to automate qPCR-based metabarcoding and amplicon sequencing workflows as laboratory automation offers numerous advantages, including increased efficiency, enhanced accuracy and reproducibility, reduced costs, faster turnaround times, and minimisation of human error. At OceanOmics Centre, we successfully developed high-throughput, precise liquid handling methods using advanced platforms to scale up metabarcoding and high-throughput amplicon sequencing. These included: transferring DNA from 96 × 1.5 mL tubes to 96-well plates in under 12 minutes; dispensing qPCR master mix into nine 384-well plates in 20 minutes; adding unique indexing primers to 384-well plates in 2 minutes; transferring DNA from three 96-well plates to three 384-well plates in 10 minutes; pooling and cherry-picking PCR amplicons from four 384-well plates in 65 minutes; and performing magnetic bead-based clean-ups for 32 samples in 80 minutes.
These workflows have been routinely used to generate high-quality amplicon sequencing data for over 4,500 eDNA samples targeting COI, MiFishU, MiFishE2, 16S, and MarVer1 metabarcoding assays, contributing to large-scale biodiversity assessments across Australian marine ecosystems between 2023 and 2025

Integrating Sustainability into Science: Lessons from the OceanOmics Centre's Sustainability Certification Journey

Ibrahim Faseeh[1], Adam Bennett[1], Adrianne Doran[1], Anna Depiazzi[1], Ebony Thorpe[1], Emma de Jong[1], Georgia Nester[1], Jessica Pearce[1], Laura Missen[1], Lauren Huet[1], Liam Anstiss[1], Sang Huynh[1], Tyler Peirce[1], Shannon Corrigan[1,2]

[1]Minderoo OceanOmics Centre at UWA, [2]Minderoo Foundation

Research laboratories are one of the most resource-intensive spaces in higher education institutions, consuming 2 to 10 times more energy than office spaces despite their relatively small footprints. Fortunately, researchers are becoming aware of the environmental impacts of research and aim to make science sustainable, and they ask themselves where to start. Typically research labs do not have the capacity to find out the most sustainable alternatives for specific processes or to identify the biggest consumers of energy and other resources by themselves.

This presentation shares our Centre's journey towards laboratory sustainability certification, highlighting key improvements and outcomes. Improvements included better waste segregation and recycling, reduced energy consumption, minimising plug load through equipment shutdown protocols, and improving resource efficiency via better inventory and purchasing practices. Key to our success was forming a local sustainability team to promote a culture of responsible laboratory practices and staff engagement.

Functional importance of a post-translational succinimide modification in a high-affinity bacterial transport protein

Ben E. Clifton[1,2], Bakhytzhan Akdavletov[2], Prashant Jain[2], Paola Laurino[2]

[1]School of Molecular Sciences, University of Western Australia, [2]Protein Engineering and Evolution Unit, Okinawa Institute of Science and Technology.

SAR11 marine bacteria are among the most abundant organisms on Earth and have a major impact on marine environments. The extraordinary ecological success of these bacteria is dependent on their ability to thrive under nutrient-poor conditions. We recently showed that SAR11 bacteria possess transport proteins with unusually high binding affinity as an evolutionary adaptation to these conditions. However, the molecular basis for high binding affinity in these proteins remained unknown. In this work, we show that spontaneous post-translational modification of a binding site residue is important for high binding affinity in the 5-oxoproline transport protein SAR11_0655. Modification of Asn269 to succinimide in the binding site of SAR11-0655 was discovered by inspection of electron density in a high-resolution crystal structure and confirmed by LC/MS-MS peptide analysis. Loss of the succinimide modification via the substitution Asn269Ala resulted in a 25-fold decrease in binding affinity from 1.2 nM to 30 nM, and a 20 °C decrease in protein thermostability. These results provide a rare example of an important role for the succinimide modification in protein stability and function, and highlight the unique adaptations of bacterial transport proteins to nutrient-poor environments.