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23RD OF NOVEMBER 2018

BRENAN HALL ST VINCENT'S HOSPITAL (MELBOURNE)

INVITED SPEAKERS

PROF. GRANT MCARTHUR

DR. MARGUERITE EVANS-GALEA

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SVI ST VINCENT'S INSTITUTE MEDICAL RESEARCH

CENTRE FOR Eye Research Australia

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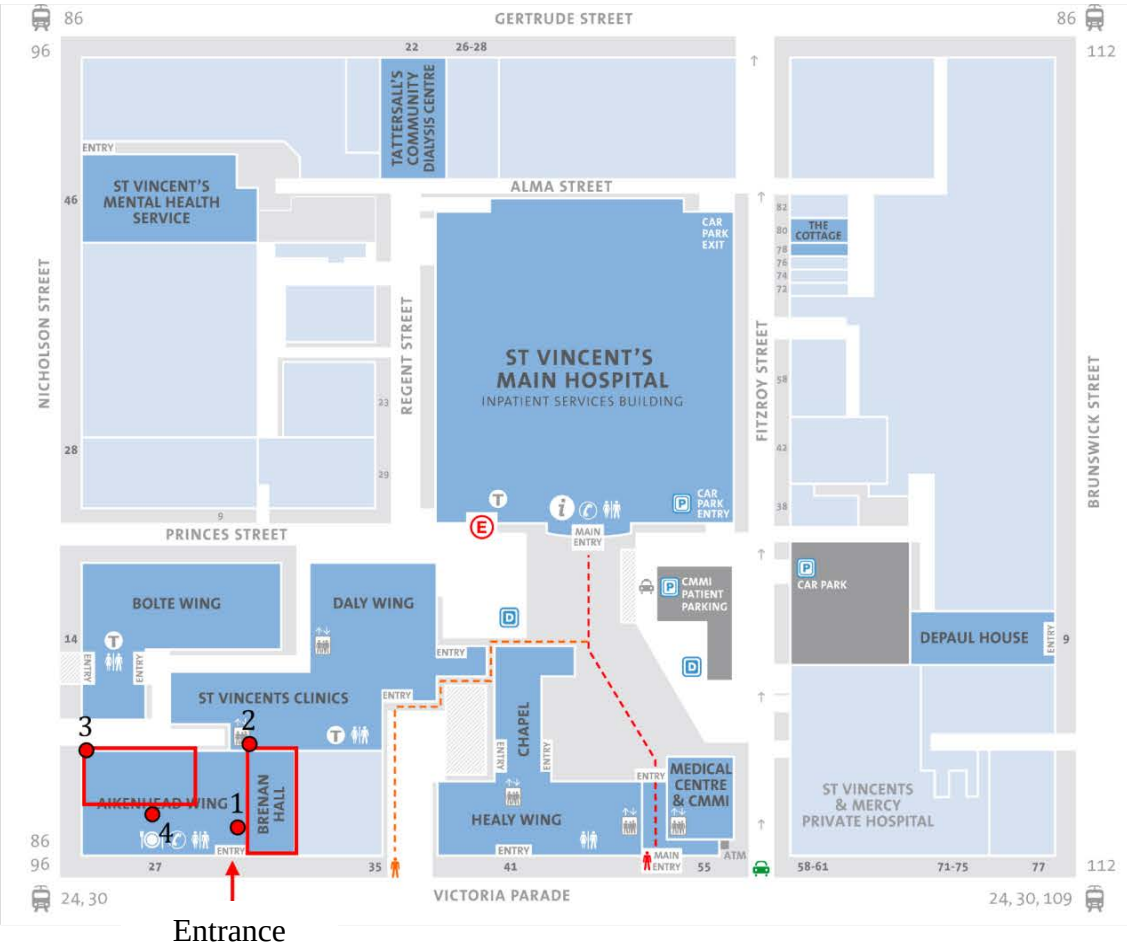
Biomed Link Location

Map – St Vincent's Hospital



Entrance

Points of Interest

1. Registration
2. Brenan Hall (Poster Area)
3. Michael Chamberlain Theatre
4. Toilets

Message from the Co-Chairs

Dear Delegates,

Welcome to Biomed Link 2018, a student conference for biomedical students to communicate their research and network with peers from Melbourne and across Australia. The Biomed Link 2018 organising committee is made up of members from the St. Vincent's Student Society, and we are proud to provide this forum for the ninth year running, made possible by our generous sponsors.

We hope that Biomed Link 2018 will provide you with opportunities to:

- Communicate your research to a broad scientific audience;
- Meet and network with peers from different research fields across Australia;
- Engage in post-presentation discussions;
- Be inspired by the career experiences of our keynote address speakers Dr. Marguerite Evans-Galea and Prof. Grant McArthur;
- Interact with the representatives from several of our generous sponsors at the trade displays.
- Find out available honours or PhD projects at St Vincent's Hospital

Moreover, we hope that the assortment of research topics covered this year by the oral and poster presentations will spark some interesting discussions amongst you and your peers and provide you with a chance to practice your communication and networking skills, while learning something in the process. We highly encourage you to take advantage of these opportunities to make the most out of this conference.

We wish you a meaningful day with us and hope that you will enjoy all that this wonderful conference has to offer.

Sean Oh and Wilson Castillo

Biomed Link 2018 Co-Chairs

Biomed Link 2018 Organising Committee

The Biomed Link 2018 conference was made possible by invaluable contributions from the committee members comprising of the following individuals:

Sean Oh

Wilson Castillo

Co-Chairs, Biomed Link 2018

Jane Xu

Marie Christensen

Diannita Kwang

Ritika Saxena

Vanessa Tsui

Patrick Lam

Please feel free to approach any of the committee members who will be wearing a white lanyard if you have any questions or require further assistance.

Biomed Link 2018 Major Sponsors

Research Training @ Eastern Hill

The University of Melbourne's Eastern Hill Academic Centre (EHAC) conducts clinical research and teaching in a hospital setting. The Centre has a wide range of research expertise including epilepsy, cancer, diabetes, cardiovascular disease, immunology, health outcomes, hearing loss and surgery.

At EHAC we aim to research and identify novel, practical solutions to significant health problems through our research and research training programs and to train the next generation of medical professionals through our Clinical School.

EHAC offers a wide range of exciting, cutting-edge translational research projects for prospective students through the following University Departments: Medicine, Surgery, Otolaryngology, Ophthalmology, and Medical Bionics.

Our Students

The Centre currently has approximately 180 students enrolled in a range of research higher degrees at the University, including MPhil and PhD. The Centre also hosts around 7 - 10 Honours students each year. Students have the opportunity to conduct their research in the Centre itself, or in affiliated medical research institutes and departments within St Vincent's Hospital. Our students also have access to cutting-edge laboratory facilities to facilitate their research in the Centre.

All support services offered to students on the main University campus are available to our students, as well as additional resources such as Research Higher Degrees coordinators, a Research Training Committee for guidance and advice, and international/ national travel allowances. We also provide laptops for new commencing PhD students and on-campus IT support.

Our Collaborators

The Centre collaborates with various institutes at both national and international levels. Some of our collaborators include St Vincent's Institute of Medical Research, O'Brien Institute, Bionics Institute, Centre for Eye Research Australia, Centre for Neural Engineering (University of Melbourne), Bio21 Institute, Monash University, University of Wollongong and St Michael's Hospital, Canada.

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http://www.medicine.unimelb.edu.au/ehac


Cancer researcher: Made at SVI
At St Vincent's Institute (SVI), our students are trained by some of Australia's leading scientists to learn the skills to help those affected by common diseases like cancer, diabetes, heart disease, arthritis, osteoporosis and Alzheimer's.
After completing her PhD with Professor Michael Parker in 2008, Dr Lorien Parker (pictured) took up a postdoctoral position at RIKEN Systems and Structural Biology Centre in Yokohama City, Japan, funded by an Overseas Biomedical Fellowship from the NHMRC. She will be back at the Institute in 2014 bringing with her all the skills and experience she garnered in her time overseas.
Lorien says, "While at SVI I was encouraged to think independently, but also to seek out assistance from other scientists within the Institute. It was this encouragement, and exposure to the wider scientific community at international conferences, which made it possible for me to be here today."
The SVI Foundation offers $5,000pa top-up scholarships to Honours and PhD students.
www.svi.edu.au/students
SVI
ST VINCENT'S INSTITUTE
MEDICAL RESEARCH

Saving sight, changing lives: Building a meaningful career

Why study at CERA?

The Centre for Eye Research Australia (CERA) is the top institute for vision research in Australia. Together with the University of Melbourne, we rank 4th in the world for academic output in Ophthalmology.[1]

We are internationally renowned for studies into the causes, diagnosis and treatment of eye diseases, vision loss and blindness.

Students, from Australia and all over the world, undertake Research Higher Degrees at CERA as part of the University of Melbourne's Eastern Hill Campus, enjoying CERA's excellent facilities in central Melbourne, recently voted the world's most liveable city for the seventh consecutive year.[2]

[1] *Centre for World University Rankings by Subject - 2017.*
[2] *The Economist, 16 Aug 2017.*

Students leading projects that change lives

- Dr William Yan, PhD student, won the 2016 Google Impact Challenge with his project that will bring eye examinations to Australians in remote and regional areas.
- Joshua Foreman was instrumental in conducting Australia's first National Eye Health Survey which revealed the scale of vision diseases.
- Karl Brown's PhD research on adult stem cells and their potential to repair damaged corneas was featured on TV, ABC radio and national print media.
- Edith Holloway is implementing a system in collaboration with Vision Australia services to manage depression in adults with low vision.

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CENTRE FOR Eye Research Australia

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Biomed Link 2018 Session Times

8:00 – 8:30	**CONFERENCE REGISTRATION**
8:30 – 8:35	**WELCOME ADDRESS** *Michael Chamberlin Lecture Theatre*
8:35 – 9:15	**PLENARY ADDRESS** *Michael Chamberlin Lecture Theatre* Dr Marguerite Evans-Galea Co-founder and CEO of Women in STEMM Australia
Session 1 **9:15 – 10:30**	**ORAL PRESENTATIONS** *SESSION 1 Michael Chamberlin Lecture Theatre* **Eleanor Norman** Investigating the role of BDNF-TRKB signaling in oligodendroglia development **Abigail Slater** Initial characterisation of immune cell entry to the brain in a mouse model of Multiple Sclerosis **Anna Trigos** Genomic drivers of the fragmentation of co-expression modules regulating multicellularity in cancer **Tu Nguyen** Generation of photoreceptors from Muller Glia using cellular reprogramming technology **Athanasios Koulis** Reduced immunosurveillance in intestinal metaplasia leads to increased risk of progression to gastric cancer
10:30 – 11:00	**MORNING TEA & POSTER PRESENTATIONS** *SESSION 1 Brennan Hall*
Session 2 **11:00 – 13:00**	**ORAL PRESENTATIONS** *SESSION 2 Michael Chamberlin Lecture Theatre* **Meg Elliott** Characterising the role of caspase-3 loss in human colorectal cancer **Wei Shem Lee** The involvement of two-hit mechanism in developmental brain malformation **Riley Morrow** Therapeutically targeting Myc in gastric cancer **Aadya Nagpal** Evaluation of Neratinib efficacy and mechanisms of resistance in a new syngeneic model of breast cancer brain metastasis **Yilin Kang** Loss of human Tim8a leads to mitochondrial dysfunction and primes cells for death

	Heidi Fettke Plasma cell-free DNA concentration and outcomes in advanced prostate cancer patients treated with androgen signaling inhibitors **Prerana Ghosh** Inhibiting PRMT5: A novel approach in melanoma treatment **Stephanie Leech** A novel neurodevelopmental disorder: synaptic defects caused by mutations in synaptotamin-1
13:00 – 14:00	**LUNCH & POSTER PRESENTATIONS** *SESSION 2 Brennan Hall*
Session 3 **14:00 – 16:00**	**ORAL PRESENTATIONS** *SESSION 3 Michael Chamberlin Lecture Theatre* **Farrah Blades** TYRO3 is a key regulator of myelin thickness in the central nervous system **Mariah Alorro** Systemic and non-tumoural STAT3 inhibition restricts gastrointestinal tumour growth in mice **Matthias Enders** Visualising MHC-II restricted response to rodent malaria **Thiago M. Steiner** CLEC9A mediated humoral immunity involved improved antigen degradation by B cells **Elizabeth Thomas** NRG1 genetic risk score predicts antisaccade and memory guided saccade latency in schizophrenia **Yau Chung Low** Human embryonic stem cell model of acylglycerol kinase (AGK) mutations in Sengers syndrome **Sherouk Fouda** Does cigarette smoking exacerbate high-fat diet induced hepatosteatosis to NASH in C57BL/6J mice **Bhavisha Patel** Using a gene expression signature to identify novel beta-cell protective compounds

Biomed Link 2018 Session Times (Continued)

16:00 – 16:30	**AFTERNOON TEA & POSTER PRESENTATIONS** *SESSION 3 Brennan Hall*
16:30 – 17:10	**KEYNOTE ADDRESS** *Michael Chamberlin Lecture Theatre* Professor Grant McArthur Executive Director (VCCC), Head, Molecular Oncology Laboratory, Senior Medical Oncologist, Peter MacCallum Cancer Centre
17:10 – 17:30	**PRESENTATION OF AWARDS** *Michael Chamberlin Lecture Theatre*
17:30 – 19:00	**CLOSING RECEPTION** *Brennan Hall*

Biomed Link 2018 Plenary Address

Plenary Address

Dr Marguerite Evans-Galea

8:35 – 9:15

Michael Chamberlin Lecture Theatre

Dr Marguerite Evans-Galea is is a scientist, executive and entrepreneur. She is the Executive Director of the Industry Mentoring Network in STEM with the Australian Academy of Technology and Engineering. She is also Honorary Fellow at the Murdoch Children's Research Institute and The University of Melbourne.

Dr Evans-Galea has led research programs in cell and gene therapy at world-leading organisations in the United States and Australia. Her research and leadership have been internationally recognised with numerous awards. Strongly committed to empowering early- and mid-career researchers, Dr Evans-Galea actively mentors students, postdoctoral fellows and faculty.

An internationally recognised advocate for women in STEMM, Dr Evans-Galea serves on the Science in Australia Gender Equity Expert Advisory Group and is co-founder and CEO of Women in STEMM Australia. She serves on the inaugural Ministerial Council for Women's Equality in Victoria and is an Ambassador for the Victorian Honour Roll of Women. Dr Evans-Galea has also represented Australia at the Asia-Pacific Economic Cooperation Women in STEMM meetings.

A strong advocate for STEMM research, education and innovation, Dr Evans-Galea has served with advisory groups in State and Federal governments, and communicates regularly on a range of science-related topics via social and mainstream media.

Biomed Link 2018 Keynote Address

Keynote Address

Professor Grant McArthur

16:30 – 17:10

Michael Chamberlin Lecture Theatre

Professor Grant McArthur is a Fellow of the Royal Australasian College of Physicians and holds a Ph.D. in Medical Biology. He is the Executive Director of the Victorian Comprehensive Cancer Centre; inaugural Lorenzo Galli Chair of Melanoma and Skin Cancers at the University of Melbourne and is a Senior Principal Research Fellow (NHMRC).

He is also Head of the Molecular Oncology Laboratory and of the Cancer Therapeutics Program, Cancer Research, and a Senior Consultant Medical Oncologist, Cancer Medicine at the Peter MacCallum Cancer Centre. He is a national and international study co-chair of a number of clinical trials of targeted therapies. His research interests include discovery of novel drug targets in cancer, targeting oncogenes, immunological effect of targeted therapies, clinical trials of targeted therapeutics, personalised medicine, melanoma, cell cycle control, metabolism and protein synthesis in cancer.

Oral Presentations

Session 1

9:15 – 10:30

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Session 2

11:00 – 13:00

Pages 22- 29

Session 3

14:00 – 16:00

Pages 30 - 37

All oral presentations will be held at the Michael Chamberlin Lecture Theatre

OP1. INVESTIGATING THE ROLE OF BDNF-TRKB SIGNALING IN OLIGODENDROGLIAL DEVELOPMENT

Eleanor Norman[1], Jessica Fletcher[1], Rhiannon Wood[1], Simon Murray[1].

[1] *Department of Anatomy & Neuroscience, The University of Melbourne, Melbourne, VIC.*

Introduction: Oligodendroglial development and myelination involves a complex system of regulatory signals; one such signal is the neurotrophin brain-derived neurotrophic factor (BDNF). BDNF acts via the TrkB receptor on oligodendrocytes, to promote myelination in the developing central nervous system (CNS). Conditional deletion of TrkB in mature oligodendrocytes was linked to hypomyelination, but also proliferation of oligodendrocyte precursor cells (OPCs), during development. This sparked suggestions that BDNF-TrkB signaling affects more than myelination. Given that TrkB gene expression is highest in OPCs, it can also be hypothesised that BDNF-TrkB signalling plays different roles throughout oligodendroglial development.

Aims: To conditionally delete TrkB at two stages of oligodendroglial development: at lineage specification, and at the mature oligodendrocyte stage.

To assess effects of conditional TrkB deletion on: myelin phenotype, oligodendroglial subpopulations, and OPC proliferation dynamics.

Methods: To delete TrkB at lineage specification, TrkB floxed ($TrkB^{fl/fl}$) mice were crossed with Olig2Cre mice. To delete TrkB in mature oligodendrocytes, $TrkB^{fl/fl}$ mice were crossed against MBPCre mice. Mice were collected at P12 and P30. To enable cell cycle calculations, double S-phase labelling with thymidine analogues was performed. Mice were anaesthetised and transcardially perfused. Spinal cords were dissected out, and processed for immunohistochemistry and electron microscopy (EM).

For immunohistochemistry, spinal cords were cryosectioned, stained with a variety of markers, and imaged on confocal and epi-fluorescent microscopes. For EM, semi-thin sections were cut and imaged via transmission electron microscopy. Myelin, oligodendroglial subpopulation density, OPC growth fraction and cell cycle lengths were all analysed in the dorsal column white matter.

Results: TrkB deletion at oligodendroglial specification resulted in diminished myelin, with qualitative analysis suggesting a reduction in the number of myelinated axons. Densities of OPCs and mature oligodendrocytes were unchanged by conditional TrkB deletion, regardless of when deletion occurred in the lineage. However, TrkB deletion in mature oligodendrocytes led to a specific and transient change in OPC proliferation dynamics, with significantly increased OPC growth fraction and cell cycle lengths observed at P12.

Conclusions: Our results provide evidence to support a pro-myelinating role for BDNF-TrkB signaling in oligodendroglia. Combined with previous observations made in conditional TrkB knockouts, our findings also suggest that TrkB expression exerts variable effects in oligodendroglia, depending on the CNS region and stage of development. That we see changes to OPC proliferation following TrkB deletion in mature oligodendrocytes, but not when TrkB is deleted earlier, suggests BDNF-TrkB signaling may indeed play different roles throughout oligodendroglial development.

OP2. INITIAL CHARACTERISATION OF IMMUNE CELL ENTRY TO THE BRAIN IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

Slater, Abigail[1]

[1] *John Curtin School of Medical Research, Canberra, ACT*

Introduction: Multiple sclerosis (MS) is a neurodegenerative disease in which immune cells enter the central nervous system (CNS) and damage the nerve insulating substance myelin. This condition leads to ascending paralysis and loss of neurological functioning. Although MS is known to cause neurodegeneration, the disease still has no cure.

Aim: Investigating immune cell population changes over the disease course and their infiltration into the CNS is essential to understanding how potential new treatments could prevent or alter MS. Based on prior research, we hypothesised that the immune cells enter the brain via the choroid plexus, the interface between the blood and cerebrospinal fluid in the ventricles.

Methods: A relapsing-remitting mouse model was used, in which immunisation with a peptide induced auto-inflammatory disease of the CNS. This model replicates the relapsing-remitting pathology of most MS sufferers, who experience periods of acute inflammation alternating with periods of remission. We performed sectioning, immunohistochemistry and confocal microscopy of the brains at control, preclinical and acute stages of disease. Flow cytometry was used to measure corresponding immune cell changes in the blood.

Results: Examination of the blood at acute disease showed significant decline in T-regulatory immune cells, suggesting their suppression as important to disease onset. Neutrophils (immune cells which are rapidly recruited to inflammatory sites) showed a preclinical increase and subsequent decrease at acute disease. This corroborates existing studies, and suggests the cells may have either died or travelled into the brain. Markers for immune and brain cell (neuron and astrocyte) populations were measured around the choroid plexus and cortex to determine the location of immune cell infiltration.

Conclusions: Preliminary results suggest the meninges, a membrane which covers the brain, as an alternate point of immune cell entry. These results will form the mechanistic basis of investigation into new treatment options for MS.

OP3. GENOMIC DRIVERS OF THE FRAGMENTATION OF CO-EXPRESSION MODULES REGULATING MULTICELLULARITY IN CANCER

Anna S. Trigos[1,2], Richard B. Pearson[2,3,4], Anthony T. Papenfuss[1,2,5], and David L. Goode[1,2]

[1]*Computational Cancer Biology Program, Peter MacCallum Cancer Centre, Melbourne, VIC Australia*

[2]*Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC Australia*

[3]*Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, VIC, Australia*

[4]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia*

[5]*Bioinformatics Division, The Walter & Eliza Hall Institute of Medical Research, Parkville, VIC Australia*

Introduction: Many hallmarks of cancer can be explained as a disruption of transcriptional networks shaped during the emergence of multicellularity, leading to tumours relying on cellular processes that date back to unicellular ancestors (e.g., cell replication, glycolysis). However, how diverse sets of genomic alterations converge to common hallmark phenotypes is currently not well understood, making the identification of true driver mutations challenging.

Aims: The aims of this study were to (1) determine how the evolutionary ages of genes are associated with module properties, (2) quantify and characterise the formation of novel, tumour-specific modules by assessing the rewiring dynamics of modules and (3) describe the role of point mutations and CNAs in altering gene co-expression and tumour module formation.

Methods: We identified modules of genes with highly correlated expression in 3494 tumour and 398 normal samples of 7 solid tumour types from The Cancer Genome Atlas, and stratified them by the predominant time of emergence in evolution of the genes in each module. We determined the role of somatic mutations in disrupting these modules by quantifying the change of localization (peripheral vs. central) of the mutated gene in regulatory networks in tumours compared to their initial localization in networks of normal tissues.

Results: We found modules dominated by either unicellular or multicellular genes were preserved between tumours and matched normal tissues, whereas modules with a mix of unicellular and multicellular genes were largely tumour-specific, and were often associated with specific recurrent point mutations and copy-number aberrations. Amplifications often led to the creation of new hubs around which tumour-specific modules formed, whereas deletions and point mutations tended to disrupt signalling between unicellular and multicellular genes in normal samples, leading to the fragmentation of modules in tumours.

Conclusion: Our results reveal how distinct genomic alterations in cancer affect gene expression by rewiring transcriptional control modules that developed during the evolution of multicellularity. This approach uncovers previously unappreciated genetic and transcriptional drivers and point to novel strategies for treatment based on mutational profiles.

OP4. GENERATION OF PHOTORECEPTORS FROM MÜLLER GLIA USING CELLULAR REPROGRAMMING TECHNOLOGY

Tu Nguyen[1], Lucy Lyujie Fang[1,2], Jafar S. Jabbari[3], Guei-Sheung Liu[1,4], Chi Luu[1], Mark Gillies[5], Alex Hewitt[1,4], Raymond Wong[1]

[1] Centre for Eye Research Australia; (Ophthalmology) Department of Surgery, The University of Melbourne, Australia, [2] Jinan University, China, [3] Australian Genome Research Facility, Melbourne, Australia, [4] Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia, [5] Save Sight Institute, Sydney, Australia

Introduction: Cellular reprogramming is an emerging technology that attracts enormous interest for its potentials in tissue regeneration. Recent advances in the field have enabled the use of master transcription factors to directly reprogram one somatic cell type to another without passing through an intermediate stem cell state. The direct reprogramming technology can be utilised to regenerate tissues lost in diseases or injuries through the conversion of endogenous support cells into the desired cell type. Photoreceptor cell death is the leading cause of many retinal diseases which can give rise to profound vision loss. As there is no effective treatment available to restore vision after photoreceptor loss, the development of a reprogramming technology to reprogram Müller glia (MG) into photoreceptors poses as a promising approach for photoreceptor regeneration.

Aim: This study aims to develop a novel approach for generating photoreceptor *in vitro*, by reprogramming MG into induced photoreceptors (iPHs).

Methods: MG to iPHs reprogramming was facilitated by a CRISPR activation (CRISPRa) system which enables multiplexed activation of endogenous genes. This platform allowed us to simultaneously activate up to 9 transcription factors and efficiently screen and optimize reprogramming cocktails for iPHs generation from a human Müller cell line, MIO-M1. Photoreceptor marker analysis was performed using RT-qPCR, immunocytochemistry, and single cell transcriptome profiling was generated using the 10X Chromium System.

Results: Using the CRISPRa technology, we were able to identify cocktails that reprogrammed MG into iPHs *in vitro.* RT-qPCR and immunocytochemistry results demonstrated the activation of the rod photoreceptor marker RHO in iPHs. To better understand the reprogramming process, we performed single cell transcriptome analysis on 17,553 MIO-M1 cells and iPHs. The results demonstrated the reprogramming of MG towards neurons, the expression of a panel of photoreceptor markers in iPHs and the presence of multiple cell populations representative of different reprogramming stages.

Conclusions: Our study has demonstrated the use of direct reprogramming to generate iPHs from MG. Successful generation of iPHs *in vitro* will provide a platform for drug screening, disease modelling, and the application of gene therapy to treat a range of retinal diseases. This method can also be applied *in vivo* in the future, offering an innovative approach to regenerate photoreceptors and alleviate blindness in diseased eyes.

OP5. REDUCED IMMUNOSURVEILLANCE IN INTESTINAL METAPLASIA LEADS TO INCREASED RISK OF PROGRESSION TO GASTRIC CANCER

Athanasios Koulis[1,2], Natasha Di Costanzo[1,2], Catherine Mitchell[1], Stephen Lade[1], David Goode[2,3], Rita Busuttil[1,2], Alex Boussioutas[1,2,4,5]

[1]Upper Gastrointestinal Translational Laboratory, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, Victoria, 3000; [2]The Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Victoria, 3010; [3]Computational Cancer Biology Program, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, Victoria, 3000; [4]Department of Medicine, Royal Melbourne Hospital, 300 Grattan Street, Melbourne, Victoria, 3050; [5]Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Victoria, 3010

Introduction: Gastric intestinal metaplasia (IM) is a relatively common premalignant lesion. There are two main subtypes of IM, the complete type (cIM) which is small intestine-like and the incomplete type (iIM) which is large intestine-like. Patients with the latter subtype have the highest risk of progression to gastric cancer.

Aims: The aim of this study was a) to characterise the immune microenvironment in IM with regards to T cells and macrophages and b) to determine whether any differences in T cell/macrophage subsets were associated with either IM subtypes.

Methods: Cases were selected on the basis of IM subtype diagnosed separately by two pathologists. Samples from patients without (IM-GC) and with concurrent gastric cancer (IM+GC) were used. As a model based on previous studies, the latter represented a more "advanced stage" of IM. Both macrophage (CD68, CD163, CD206, IRF8, PDL1, AE1AE3 and dapi) and T cell (CD3, CD4, CD8, CD10, FOXP3, AE1AE3 and dapi) multiplex immunohistochemistry panels (OPAL) were optimised for IM tissue. Quantitative analyses were done with Inform software (Perkin Elmer) and spatial location analyses with RStudio ("phenoptr" R package).

Results: The majority of macrophages in cIM and iIM in both IM-GC and IM+GC cohorts had an M2 phenotype (CD68+CD163+ and CD68+CD163+CD206+IRF8-/+). Only a small number of M1 macrophages (CD68+IRF8+) were identified in these tissues. In the IM-GC cohort, patients with cIM had significantly more helper and regulatory T cells (both p=0.036, Mann-Whitney test) than patients with iIM. In the IM+GC cohort, there were significantly more T cells in cIM than in iIM (p=0.0162, Mann-Whitney test) overall. There were more helper as well as more cytotoxic T cells in cIM than in iIM (both p=0.0162, Mann-Whitney test). The results of spatial location analyses (nearest neighbour, touching cell pairs and cells within a radius of 25 microns) reflected the presence or absence of quantitative differences in both cohorts.

Conclusion: Our results show that 1) M2 macrophages are highly prevalent in IM irrespective of subtype and cohort, 2) there are quantitative differences in T cell subsets between cIM and iIM and 3) the quantitative differences are reflected in cell to cell spatial relationships. The reduced number of cytotoxic T cells in iIM of the IM+GC cohort leads us to postulate that reduced immunosurveillance plays a key role in IM progressing to GC. This study is still ongoing and we hope our findings will allow improved patient stratification.

OP6. CHARACTERISING THE ROLE OF CASPASE-3 LOSS IN HUMAN COLORECTAL CANCER

Meg J Elliott[1,2], Anuratha Sakthianandeswaren[1,2], Dmitri Mouradov[1,2], Oliver M Sieber[1,2,3].

[1] *Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.*

[2] *Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia.*

[3] *Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia.*

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer in Australia, with ~15,000 new cases annually. Despite improvements in treatment, CRC remains the second leading cause of cancer-related mortality. Genome sequencing projects have revealed the landscape of genetic alterations found in human CRC, highlighting multiple novel candidate driver genes. The caspase-3 gene on chromosome 4q has been identified as recurrently deleted in CRC, however the contribution of caspase-3 loss to cancer development is unknown. Caspase-3 is a cysteine protease involved in the execution phase of apoptosis, responsible for the cleavage of structural and DNA repair proteins, leading to the cell demise.

Aims: We aim to determine the prevalence and mechanisms of caspase-3 loss in CRC, understand the functional consequences of caspase-3 loss and perform a drug response screen in CRC cell lines.

Methods: To define the prevalence of caspase-3 loss in CRC we have performed somatic copy number aberration (SCNA) studies using CRC cell line, in-house patient sample and The Cancer Genome Atlas (TCGA) data. Messenger RNA (mRNA) and protein expression were also assessed in CRC primary cancer specimens and cell lines.

We also generated caspase-3 wild type (WT), heterozygous knock-out (KO) and homozygous KO cell lines using CRISPR/Cas9 technology and mutants were validated using Sanger sequencing and Western blot. Phenotypic and functional consequences of caspase-3 loss were evaluated in relation to cell viability, caspase-3/7 activity, clonogenic potential, proliferation, cell cycle, cell death and drug responses.

Results: Our SCNA studies in CRC indicate deletions of caspase-3 occur in ~10% of cases. Point mutations are rare (3/82 CRC cell lines), however, mRNA and protein expression data indicate loss of caspase-3 expression in up to 20% of caspase-3 wild-type tumours. Isogenic cell line data have revealed that heterozygous and homozygous KO cells exhibit increased cell viability and elevated clonogenic potential compared to the WT cell lines. Cell cycle analyses revealed a significantly greater number of G2/M cells in KO cell lines when compared to WT. Heterozygous and homozygous KO cell lines also exhibited increased resistance to treatment with BH3 mimetic drugs.

Conclusions: CRC is a major malignant disease worldwide, with an urgent need for improved treatments. Determination of the molecular mechanism by which caspase-3 loss promotes carcinogenesis will advance our understanding of fundamental CRC biology. High-throughput drug screening studies may highlight novel diagnostic and therapeutic avenues to improve patient outcomes.

OP7. THE INVOLVEMENT OF TWO-HIT MECHANISM IN DEVELOPMENTAL BRAIN MALFORMATION

Wei Shern Lee[1,2], Sarah Stephenson[1,2], Richard Leventer[3], Simon Harvey[3], Wirginia Maixner[3], Paul Lockhart[1,2]

[1]*Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC, Australia*

[2]*Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia*

[3]*Department of Neurology, Royal Children's Hospital, Parkville, VIC, Australia*

Introduction: The development of cerebral cortex involves multiple steps of neuronal proliferation, migration, and differentiation. Interruption to these processes during development can cause congenital brain malformation including focal cortical dysplasia (FCD). Characterised by focal disruption of cortical layering and the presence of dysmorphic neurons, FCD is a leading cause of drug-resistant childhood epilepsy that often requires surgical intervention for seizure control. Recent studies have reported germline mutations affecting *MTOR* pathway genes in FCD patients. However, it is unclear how a germline mutation can cause focal brain lesion that only affects a small part of the brain.

Aim: To understand the genetic basis and pathogenesis of FCD using a unique archive of patient-derived, resected brain tissues collected over an 8-year period.

Methods: We investigated one FCD case with a known germline mutation identified through previous study. Genomic DNA samples were extracted from resected brain tissues and peripheral blood for deep sequencing (>500x depth) to uncover rare somatic mutations. Following the identification of somatic mutation, the precise mutation load (typically <5% allele frequency) was determined by droplet digital PCR. Epileptic activity in the brain was assessed during surgery using electrocorticography. Immunohistochemical and stereological analyses were used to assess the histopathology of formalin-fixed, paraffin-embedded brain specimens.

Results: In one case with a known heterozygous germline mutation in *DEPDC5*, we identified a low allele frequency (4%) somatic mutation affecting the same gene. The somatic mutation is located on the opposite allele to the germline mutation, and is only found in the affected brain tissue but not in the peripheral blood. This suggests that a brain-specific, bi-allelic inactivation of *DEPDC5* is likely contributing to FCD. We further showed a 'mutation gradient' in the affected cortex, in which different levels of somatic mutation load were found across five brain regions. Notably, the region with the highest mutation load was found to be located at the origin of epileptic activity. Further analysis showed that this region contains the most severe histopathology and the highest number of dysmorphic neurons.

Conclusions: Our data suggest that a 2-hit mechanism is involved in the development of FCD. Future work will explore the involvement of 2-hit mechanism in nine additional FCD cases with known germline mutations. Our results offer novel insights into the genetic basis and pathogenesis of FCD, which will be highly applicable to the broader research in epilepsy and brain development.

OP8. THERAPEUTICALLY TARGETING MYC IN GASTRIC CANCER

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Introduction: Gastric cancer remains deadly due to a lack of effective therapeutic options and late diagnosis of disease. Myc is well described as being critical for gastric tumour development and progression. Various studies have confirmed the overexpression of Myc in primary gastric cancers. Hyperactivation of inflammatory signalling cascades, including the Janus kinase/Signal transducer and activator of transcription 3 (Jak/Stat3), is crucial for gastric cancer development and can cause the overexpression of Myc, however its pro-tumourigeneic role in this setting remains poorly understood.

Aim: Previous findings from our lab using the $Gp130^{FF}$ mouse model, which spontaneously develop gastric adenomas through Interleukin-11 dependent hyperactivation of Stat3, identified that Myc expression is significantly raised within these tumours. Further, systemic loss of a single Myc allele within this model significantly decreased tumour burden. Here, we investigate the cellular requirement for Myc in these gastric tumours by genetically reducing Myc specifically within epithelial cells. As well, we explore the therapeutic benefit of diminishing Myc transcriptional activity through the use of the small-molecule inhibitor IBET-151.

Methods: $Tff1^{CreERT2}$;Myc^{flox};$Gp130^{FF}$ compound mutant mice were treated with tamoxifen and/or IBET-151 and stomachs collected. Tumour weight was recorded and tissues subjected to quantitative real-time polymerase chain reaction, Western-blot, and immunohistochemical analysis to monitor the activation of Jak/Stat3 signalling.

Results: A genetic reduction of Myc specifically within the gastric epithelium significantly reduced tumour growth, as well as activation of the Jak/Stat3 signalling pathway determined by a decrease in phosphorylated-Stat3. Therapeutic intervention with IBET-151 could also significantly reduce tumour growth, which was associated with a significant decrease in Myc mRNA expression, demonstrating its therapeutic benefit in this gastric cancer mouse model.

Conclusion: Our findings indicate that excessive Myc expression in the epithelial compartment may contribute to gastric cancer initiation and maintenance. Thus, Myc represents a promising therapeutic target for the treatment of gastric cancer.

OP9. EVALUATION OF NERATINIB EFFICACY AND MECHANISMS OF RESISTANCE IN A NEW SYNGENEIC MODEL OF BREAST CANCER BRAIN METASTASIS

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Introduction: Human epidermal growth factor receptor-2 (HER2)-targeted therapies effectively control systemic disease but resistance to treatment is common and up to 50% of patients progress to incurable brain metastases. Previous studies have shown that □v□3 integrin receptors contribute to the progression of brain metastases and resistance to receptor tyrosine kinase inhibitors (TKIs). However, therapies co-targeting these receptors have not been evaluated due to the lack of relevant preclinical models of brain-metastatic HER2 breast cancer.

Aims: The aims of this project are to i) characterise a novel syngeneic mouse model of HER2 breast cancer brain metastasis (TBCP-1) and ii) evaluate its response to HER2 and β3 integrin inhibitors alone or in combination.

Methods: TBCP-1 cells were derived from a spontaneous mammary tumour in a Balb/c mouse and characterised for hormone receptors and HER2 expression by immunoblotting or by immunohistochemistry in tumours. TBCP-1 metastasis was analysed *in vivo*. Resistant variants (TBCP-1NR) were developed by continuous exposure to the HER2 inhibitor neratinib and RNAseq used to reveal transcriptomic changes. Response to HER2 and integrin inhibitors was evaluated in *in vitro* proliferation inhibition assays. Efficacy of neratinib *in vivo* was evaluated against late stage brain metastatic disease or in a neoadjuvant setting.

Results: TBCP-1 cells, tumours or brain metastases lack hormone receptors but naturally express high levels of HER2. Accordingly, TBCP-1 cell proliferation is reduced by HER2 inhibitors, lapatinib or neratinib, but not by anti-oestrogens, indicating phenotypic and functional similarities to human HER2 breast cancer. Importantly, TBCP-1 tumours give rise to a high incidence of spontaneous brain metastases from mammary tumours or experimental brain metastases following intra-cardiac inoculation. We show for the first time that neoadjuvant neratinib potently inhibits tumour growth and spontaneous metastasis to brain and other organs and significantly prolongs disease free survival. Furthermore, we demonstrate that neratinib, but not other TKIs, induces cell death by ferroptosis in TBCP-1 or in human SKBR3 cells but fails to induce this response in TBCP-1NR resistant variants. Pharmacological inhibition of αvβ3 integrin synergistically inhibits proliferation and restores sensitivity to neratinib in resistant TBCP-1NR cells *in vitro*.

Conclusions: The TBCP-1 is the only model that fully recapitulates the spontaneous spread of HER2 breast cancer to brain in immune-competent hosts and provides a unique tool to identify novel therapeutics and biomarkers.

OP10. LOSS OF HUMAN TIM8A LEADS TO MITOCHONDRIAL DYSFUNCTION AND PRIMES CELLS FOR DEATH

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Introduction: Mutations in *TIMM8A* cause Mohr-Tranebjaerg syndrome (MTS), an X-linked disease characterised by a deafness, dystonia, paranoia, blindness and mental deterioration. MTS was the first disease linked to mitochondrial protein import dysfunction, as hTim8a shares homology with the yeast intermembrane space chaperone Tim8. However, the pathomechanism underlying MTS, and indeed the function of hTim8a remains unresolved.

Aims: This study aims to establish the function of hTim8a with a hope to uncover the pathomechanism underlying MTS.

Methods: To define function of hTim8a in mitochondrial biology, we undertook CRISPR/Cas9-genome editing of *TIMM8A* in non-neuronal HEK293 and the neuronal-like SH-SY5Y cells. Quantitative proteomics and metabolomics analyses were performed to uncover alteration of mitochondrial proteome and metabolome upon hTim8a loss. Given the unique association of MTS with neuronal cell death, mitochondrial and cellular heath assays were also utilised to identify potential dysfunctions eliciting the observed cell death.

Results: We identified cell-type specific consequences on the mitochondrial proteome due to depletion of hTim8a. In both HEK293 and SH-SH5Y cell lineages, these proteomic perturbations result in down-stream mitochondrial dysfunction, in particular mitochondrial membrane depolarisation, elevation of reactive oxygen species (ROS) and Tricarboxylic acid (TCA) cycle perturbations. Mitochondrial dysfunction in these cells amplifies the levels of mitochondrial cytochrome *c*, priming these cells for apoptotic cell death. Interestingly, loss of hTim8a specifically affects the complex IV biogenesis in neuronal cells, suggesting that enhanced sensitivity of neuronal cells to cell death underlies MTS.

Conclusion: Our work suggests that hTim8a has cell specific functions, and in neuronal cells, loss of hTim8a influences Complex IV biogenesis. Our research provides insight into the molecular features eliciting and inducing the neuronal cell loss, revealing information into the neurological pathology of MTS and also identify new molecular target that can be considered for therapeutic intervention.

OP11. PLASMA CELL-FREE DNA CONCENTRATION AND OUTCOMES IN ADVANCED PROSTATE CANCER PATIENTS TREATED WITH ANDROGEN SIGNALLING INHIBITORS

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Introduction: Recent data has demonstrated the utility of baseline plasma cell-free DNA (cfDNA) concentration as an independent prognostic indicator for metastatic castration-resistant prostate cancer (mCRPC) patients receiving systemic chemotherapy. However, the clinical utility of this biomarker is still unknown in the androgen receptor signalling inhibitor (ARSI)-treated population.

Aims: Our objective was to correlate baseline cfDNA concentration and clinical outcomes in patients commencing ARSI for mCRPC.

Methods: Blood for cfDNA analysis was prospectively collected from 42 mCRPC patients prior to commencing abiraterone or enzalutamide. Evaluable patients were required to have a minimum of 12 weeks follow-up. cfDNA was extracted from plasma using the QIAamp circulating nucleic acid kit as per the manufacturer's protocol, then quantified using fluorescence spectrometry (Qubit). Univariable analyses was conducted using logistic regression to determine if there was an association between baseline cfDNA concentration (expressed as $\log_{10}$ concentration) and prostate-specific antigen (PSA) response. In addition, Cox proportional hazard models tested for associations between cfDNA concentration (plus other baseline prognostic variables) and the composite endpoint of clinical/radiological progression-free survival (clin/rPFS). All p-values <0.05 were considered significant.

Results: The median baseline cfDNA concentration in this cohort was 8.2ng/ml (IQR 4.7 - 14.9ng/ml). Median follow-up was 9.2 months. Baseline $\log_{10}$ cfDNA concentration was not associated with a confirmed PSA response (odds ratio [OR] = 0.4; p=0.2). Median clin/rPFS was not reached. In univariate analyses, baseline $\log_{10}$ cfDNA concentration significantly correlated with shorter clin/rPFS (hazard ratio [HR] = 3.1; 95% CI: 1.2-7.7; p=0.02). Other baseline prognostic factors correlating with shorter clinical/rPFS included $\log_{10}$ LDH concentration (p=0.002) and $\log_{10}$ neutrophil-lymphocyte ratio (p=0.008).

Conclusions: In mCRPC patients commencing ARSI, higher baseline cfDNA concentration was associated with shorter clinical/rPFS, and a non-significant trend towards lower PSA response rates. Baseline cfDNA may be a potential independent prognostic biomarker for patients commencing ARSI, but validation will require greater patient numbers and ongoing data maturation. Future directions include cohort expansion, and analysis of on-progression samples.

OP12. INHIBITING PRMT5: A NOVEL APPROACH IN MELANOMA TREATMENT

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Introduction: Melanoma is the deadliest form of skin cancer. In majority of melanoma, the tumour suppressors, RB1 and p53 are inactivated. Phosphorylation and inactivation of RB1 is due to increased CDK4 activity, which is a function of activating mutations in RAS/RAF/MEK/ERK (MAPK) signalling and loss of p16, the negative regulator of CDK4. Most melanomas harbour wildtype p53 that is inactivated via high MDM4 expression. Recent studies in the lab have revealed that palbociclib, a CDK4 inhibitor, not only reactivated RB1 but also activated p53. Activation of p53 was via palbociclib mediated inhibition of protein arginine methyltransferase 5 (PRMT5) which in turn decreased MDM4. Additionaly, combining palbociclib with a PRMT5 inhibitor potently inhibted cell proliferation. Considering CDK4 is activated by the MAPK pathway we hypothesized that inhibition of the MAPK pathway will decrease PRMT5 activity and activate p53 and as such the combination of a MAPK inhibitor with a PRMT5 inhibitor would lead to a robust response.

Aims: To assess the effects of a BRAF inhibitor on PRMT5 activity, MDM4 expression and p53 activation. To assess if the combination of BRAF inhibitor plus PRMT5 inhibitor leads to a robust inhibition of cell proliferation

Methodology: A375 human melanoma cells were harvested for protein and RNA after a 6-day treatment with $1\mu M$ of vemurafenib (BRAF inhibitor), $0.5\,\mu M$ of GSK3326595 (PRMT5 inhibitor currently in clinical trials) or their combination. Western Immunoblot analysis was performed to observe changes in levels of dimethylation of histone 3 arginine 4 (marker of PRMT5 activity), MDM4 protein, total p53 and p21. Conventional PCR and gel electrophoresis were conducted to assess changes in the levels of the functional, full length MDM4 (MDM4-FL) and the unstable MDM4 (MDM4-S) transcripts. Proliferation assays were conducted on A375 cells treated with $1\mu M$ of vemurafenib, $0.5\,\mu M$ of GSK3326595 or their combination using an IncuCyte Zoom. Cell confluency was assessed every 24 hours following drug treatment.

Results: Vemurafenib as a single agent and in combination with GSK3326595, decreased PRMT5 activity and MDM4 expression while promoting p53 activation. The combination also reduced expression of the MDM4-FL isoform. Additionally, combining vemurafenib with GSK3326595 potently inhibited A375 cell proliferation and was better than the single agents.

Conclusion: Considering MDM4 is frequently overexpressed in melanoma and one of the mechanisms behind suppression of p53 activity, this combination serves as a promising therapeutic approach that can reactivate p53 pathway leading to inhibition of cell proliferation.

OP13. A NOVEL NEURODEVELOPMENTAL DISORDER: SYNAPTIC DEFECTS CAUSED BY MUTATIONS IN SYNAPTOTAGMIN-1

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Introduction: Effective communication in the brain is required for all functions of life and is dependent on the precise control of neurotransmitter release from presynaptic nerve terminals. This process is orchestrated by complex presynaptic machinery, including the integral synaptic vesicle protein, synaptotagmin-1 (syt-1), which is responsible for fast, synchronous neurotransmitter release in central neurons. Syt-1 is essential for the fusion of synaptic vesicles with the nerve terminal membrane during exocytosis, the retrieval of synaptic vesicles during endocytosis, and for clamping spontaneous neurotransmitter release. Human mutations identified in the gene that encodes syt-1 result in a novel neurodevelopmental disorder, producing severe cognitive and behavioural symptoms.

Aims: This study aims to determine the effect of human mutations on various functions of syt-1 in the synaptic vesicle cycle, and to differentiate phenotypes between each mutant variant.

Methods: Primary embryonic mouse hippocampal cultures were transfected with syt-1 mutant variants and either fixed and immunolabelled, or used for live cell imaging experiments. Fixed cells were used to determine protein expression levels and localisation at nerve terminals, and co-localisation with other presynaptic proteins. Live cell imaging experiments were carried out using pHluorins, which are pH-sensitive reporter proteins that report exocytosis and endocytosis. pHluorin imaging was used to observe surface expression of syt-1 mutant variants, effects on spontaneous neurotransmitter release, and synaptic vesicle pool size and composition.

Results: All syt-1 mutant variants were expressed at the same level as wild type syt-1, at roughly 2-fold of untransfected control neurons within the same culture. All variants were correctly localised to presynaptic nerve terminals, and further, showed similar surface expression to WT, indicating accurate targeting to synaptic vesicles. Syt-1 mutant variants showed differential effects on both rate and amount of spontaneous release, and the proportion of the total vesicle pool recruited during spontaneous release. Changes in the percentage of synaptic vesicles recruited during spontaneous release also correlated with changes in the capacity of neurons transfected with the mutant variants to undergo evoked release after a 10-minute period of spontaneous release.

Conclusions: Syt-1 mutant variants show mutation-specific effects on spontaneous release, indicating that the type of mutation harboured determines the precise defects on synaptic vesicle cycling. The pathogenic mechanisms underlying neurological dysfunction may differ between mutations in individuals with syt-1-related neurodevelopmental disorder.

OP14. TYRO3 IS A KEY REGULATOR OF MYELIN THICKNESS IN THE CENTRAL NERVOUS SYSTEM

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Introduction: Multiple sclerosis (MS) is an autoimmune, demyelinating disease of the central nervous system (CNS). Major deficits arise in MS patients due to an inability to adequately remyelinate following CNS insults, resulting in prolonged axonal exposure and neurodegeneration. Tyro3, Axl and Mertk (TAM) receptors have been implicated in MS susceptibility and pathophysiology. Previously, our laboratory has shown that Tyro3 regulates myelin thickness within the optic nerve and rostral region of the corpus callosum (CC) of adult mice.

Aims: This study aimed to comprehensively analyze the influence of the Tyro3 receptor on myelination and demyelination in the rostral and caudal regions of the CC using the cuprizone mouse model.

Methods: Demyelination and remyelination were assessed using the cuprizone model in both Tyro3$^{-/-}$ and WT C57Bl/6 background mice via the ingestion of 0.2% (w/w) cuprizone (bis-cyclohexanone oxaldihydrazone). Total myelinated axons and myelin thicknesses were determined using transmission electron microscopic imaging and ImageJ measurements. Microglia, mature oligodendrocytes (mOL) and oligodendrocyte precursor cell (OPC) densities were assessed via fluorescent immunohistochemical staining and cell counting techniques. Finally, statistical analyses were performed in GraphPad PRISM 7.

Results: We show that the rate of demyelination increased in the Tyro3$^{-/-}$ mice, with a 4-fold reduction in myelinated axons in the rostral CC. In contrast, no differences in the number of myelinated axons were observed in the caudal CC. In addition, we found that the absence of Tyro3 was associated with a reduction in myelin thickness both in unchallenged mice and following recovery from experimentally-induced demyelination. We show that this reduction in myelin thickness was due to a decrease in the number of myelin lamellae surrounding axons with, on average, three fewer lamellae following recovery from demyelination. Furthermore, thinner myelin occurred independent of influences upon OPC maturation or mOL/microglial densities.

Conclusions: As the hypomyelinated phenotype observed in the absence of Tyro3 occurred independently of any influence upon OPC maturation, or density of oligodendrocytes or microglia we suggest that the primary effect of Tyro3 is upon the radial expansion of myelin rather than alterations to glial densities. The loss of Tyro3 leads to a reduction in the number of myelin lamellae on axons and is therefore most likely a key component of the regulatory mechanism by which oligodendrocytes match myelin production to axonal diameter, a mechanism which remains elusive in the myelin field to date.

OP15. SYSTEMIC AND NON-TUMOURAL STAT3 INHIBITION RESTRICS GASTROINTESTINAL TUMOUR GROWTH IN MICE

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Stat3 is a transcription factor that is important for physiological processes such as development, immunity and inflammation. It is found to be hyper-activated in a number of different cancers, including gastric and colon cancer. Intrinsic aberrant Stat3 activation within tumour cells has been shown to drive pro-tumorigenic functions such as sustaining proliferation, resisting cell death and avoiding immune destruction. Interestingly Stat3 activation has also been demonstrated to manipulate tumour-infiltrating immune cells to exhibit pro-tumorigenic activities that support tumour development and growth. Therefore, Stat3 and its associated pathways present promising targets for cancer therapy. Although multiple Stat3 targeting inhibitors have reached clinical trials, these inhibitors require further validation for their specificity and clinical potential. To overcome this, we utilized genetic mouse models that allow for the conditional manipulation of Stat3 levels in mice challenged with gastric or colon cancer.

This project utilizes the novel CAGSrtTA3;Stat3.1348 (shStat3) mouse, which exploits short hairpin (sh) RNAi technology to allow for the inducible and reversible reduction of Stat3. The shStat3 mice were crossed with the $gp130^{Y757F/Y757F}$ ($gp130^{F/F)}$ mutant gastric cancer mice. Additionally, to study the effects of Stat3 reduction in the non-tumoural compartment, the shStat3 mice were subcutaneously engrafted with MC38 murine colon cancer cells.

Characterisation of the shStat3 mice showed effective reduction of Stat3 in the RNA and protein level of various cells types and organs. Decreasing Stat3 in the shStat3;$gp130^{F/F}$ gastric cancer model resulted in significantly reduced tumour burden. Comparably, the tumour growth of the shStat3-MC38 allografts was restricted when Stat3 was reduced among the non-tumoural cells alone. Analysis of these tumours revealed the possible role for the monocytic ($Ly6C^{+}Ly6G^{-}$) myeloid derived suppressor cells in Stat3-reduction mediated anti-tumour responses.

This project presents a unique inducible model that allows for the controlled, reversible and specific manipulation of Stat3 in vivo. Using this model we have generated evidence supporting the therapeutic value of specifically targeting Stat3 in the context of gastrointestinal cancers. Our studies have also highlighted the importance of considering the tumour microenvironment in the development of future Stat3 specific inhibitors.

OP16. Visualizing MHC-II restricted response to rodent malaria

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Introduction: Malaria is caused by different *Plasmodium* species that can infect a variety of animals including humans and rodents. Upon infection of the host, the parasites traverse through a complex life cycle, including a blood stage and a liver stage of infection. Even though the host's immune response towards each of these stages is incompletely understood, CD4 T cell responses are known to play an important role in mediating immunity towards *Plasmodium* infection throughout the parasite's life cycle.

Aims: This project aims to examine the evolution of an MHC II-restricted response to a novel MHC II-restricted epitope during *Plasmodium* infection in B6 mice and to characterise the protective capacity of this specificity.

Methods: To track CD4 T cell mediated immune responses, we made use of a recently discovered MHC II-restricted epitope that is expressed by both rodent and human *Plasmodium* species and is presented by the MHC II IA^b haplotype of B6 mice. This epitope, abbreviated as YYI, is recognized by the PbT-II T cell receptor transgenic T cells. As a potential vaccination strategy, anti-Clec9A antibody was genetically fused to the YYI epitope, and the Clec9A-targeted epitope then used to prime immunity. This led to extensive expansion of PbT-II cells, greater than seen in response to authentic *Plasmodium* blood-stage infection.

Results: In an initial step, GFP-expressing PbT-II transgenic T cells were injected into naïve B6 mice that were primed with Clec9A-YYI and then infected with PbA liver- or blood- stage parasites. 2-Photon intravital imaging of the liver revealed formation of PbT-II cell clusters 24 hours after liver stage parasite infection. These clusters mostly contained cells with a patrolling phenotype and dissipated by 48h post- infection. While transfer of naïve PbT-II cells did not lead to improvement of the outcome of liver stage infection, *in vitro* differentiated and activated Th1 PbT-II cells could reduce the blood parasite burden and prevent pathology after liver stage infection.

Conclusions: These results highlight the potential of PbT-II cells to mediate protection against *Plasmodium* infection. Future studies will determine whether anti-Clec9A-YYI has potential as a malaria vaccine and examine its mechanistic basis for protection.

OP17. CLEC9A MEDIATED HUMORAL IMMUNITY INVOLVES IMPROVED ANTIGEN DEGRADATION BY B CELLS

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Introduction: Induction of potent humoral responses is a feature of most successful vaccines. It is known that Dendritic Cells (DCs) support humoral immunity by inducing $CD4^+$ T Helper Cells (T_{FH}), however their role in supporting B cell activation has been poorly assessed. The fact that (Ag) targeted to Clec9A on $CD8^+$ conventional DCs (cDC1) induces strong humoral responses even in the absence of adjuvant makes it a target to be exploited. We have recently observed that Ag targeted to Clec9A is retained in its native form on the surface of cDC1, which allowed direct delivery of Ag to B cells. We have also described that B cell activation is enhanced by prolonged exposure of Ag anchored to Clec9A, enabling B cell migration, which was essential to support T_{FH} development. We hypothesised that improved Ag capture by B cells is also fostered when this is bound to Clec9A.

Aim: Compare Ag capture by B cells when Ag is displayed on Clec9A by cDC1 versus soluble Ag.

Material and Methods: We made use of OB1 cells, which recognize an OVA epitope. We engineered an anti-Clec9A antibody (aClec9A-OVA_{FGD}) that contains genetically fused OVA epitopes recognized by OB1 cells. OB1 cells were then transferred into C57Bl6 mice, which were then immunized with either fluorescently labelled aClec9A-OVA_{FGD} or isotype-OVA_{FGD}.

Results: We found that Clec9A mediated Ag-presentation to B cells does not lead to increased Ag uptake by B cells, but to improved Ag degradation. We also observed that MHC II expression in B cells is only significantly upregulated upon Clec9A-targeting, suggesting that improved Ag degradation potentiates presentation by B cells to T_{FH}. Thus, targeting Ag to Clec9A on cDC1 represents a new mechanism for B cell activation and humoral immunity, which can be exploited by novel vaccination approaches.

Conclusion: The mechanism involved with a potent humoral response when Ag is displayed on Clec9A not only involves efficient B cell activation and T_{FH} priming but also efficient Ag degradation.

OP18. NRG1 GENETIC RISK SCORE PREDICTS ANTISACCADE AND MEMORY-GUIDED SACCADE LATENCY IN SCHIZOPHRENIA

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Introduction: Neuregulin-1 (NRG1), involved in neuronal development, migration, myelination and synaptic plasticity, has been identified in numerous linkage association studies as a promising candidate gene for schizophrenia risk. Several single nucleotide polymorphisms (SNPs) in the NRG1 gene have been associated with schizophrenia and cognitive deficits such as saccadic (eye movement) deficits. Previous research investigating NRG1 and saccadic performance has looked at single SNPs. However, genetic liability for schizophrenia is multifactorial, with contributions of multiple risk variants. Therefore, analysis of genetic risk scores may better capture the genetic contribution to cognitive performance in schizophrenia compared to single SNP analysis, which have small effect sizes.

Aims: To investigate whether the genetic risk score for NRG1 predicts saccadic performance in patients and controls.

Methods: One-hundred and sixty-six Caucasian participants (44 patients with schizophrenia/schizoaffective disorder and 122 healthy controls) completed the antisaccade and memory-guided saccade tasks, which engage spatial working memory and inhibition processes. The variables analysed for both saccade paradigms were error rate, latency (ie. reaction time) and gain (ie. spatial accuracy). Participants were also genotyped for five NRG1 SNPs; rs10503929, rs3924999, rs2466058, rs35753505 and rs6994992. Genetic risk scores were created by assigning one point to the score for each risk allele that a participant had at each of the five polymorphic results.

Results: Antisaccade and memory-guided saccade latency and error rate were significantly different between patients and controls ($p<0.001$). Both antisaccade and memory-guided saccade gain did not significantly differ between the two groups. In patients, the NRG1 risk score significantly correlated with antisaccade latency ($p=0.037$, $r=0.389$) and explained 15.1% of the total variance of the model. The NRG1 risk score also significantly correlated with memory-guided saccade latency ($p=0.018$, $r=0.435$) and explained 18.9% of the total variance of the model. There was no relationship between NRG1 risk score with antisaccade or memory-guided saccade error rate or gain in patients. There was no relationship between NRG1 risk score with antisaccade or memory-guided saccade latency, error rate or gain in controls.

Conclusion: This is the first study to use risk scores to observe the relationship between NRG1 and eye movement performance. Preliminary findings indicate that a risk score derived from NRG1 SNPs significantly predicts antisaccade and memory-guided saccade latency in schizophrenia. This identifies NRG1 as a potential candidate gene for cognitive impairment in schizophrenia. This finding also supports the use of aggregate genetic risk scores to investigate multifactorial disorders.

OP19. HUMAN EMBRYONIC STEM CELL MODEL OF ACYLGLYCEROL KINASE (*AGK*) MUTATIONS IN SENGERS SYNDROME

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Aim: Mitochondria play an important role in cellular processes including energy generation, cellular signaling, calcium regulation and programmed cell death. These double membrane organelles require appropriate protein and lipid compositions to function properly. Acylglycerol kinase (AGK) is a key component of mitochondrial lipid metabolism. More recently, we discovered that AGK is also involved in mitochondrial protein import. AGK stabilises the TIM22 protein import complex as well as support the integration of essential carrier proteins within the mitochondrial inner membrane. Mutations in *AGK* results in Sengers syndrome – a potentially fatal mitochondrial disease – symptoms of which include hypertrophic cardiomyopathy, skeletal myopathy, lactic acidosis, and congenital cataracts. This project aims to model and investigate the molecular and cellular pathogenesis underlying Sengers syndrome using human embryonic stem cells (hESCs) due to its dual role in maintaining mitochondrial function.

Methods: Using CRISPR/Cas9 gene editing technology, we have successfully deleted the *AGK* gene in hESCs. These cell lines were screened and validated by DNA sequencing and immunoblot experiments. Following this, selected clones were differentiated into cardiomyocytes to replicate the pathogenesis of Sengers syndrome. Further functional analyses are currently being conducted to determine the effects of disrupted mitochondria within these cardiomyocytes. These include: 1) calcium imagining to determine changes in the calcium handling ability of the cardiomyocytes, 2) looking at any alterations in mitochondrial structure by electron microscopy, and 3) changes to the protein compositions in the cell as a result of the mutations.

Results: DNA sequencing and cDNA studies identified mutations causing a frameshift and premature stop codon, resulting in truncated AGGK proteins being encoded. In hESCs with mutations, the TIM22 complex was not detected by immunoblotting. Other preliminary results indicate multiple defects in cardiomyocytes derived from the mutant cell lines. Mutants display an irregular beating pattern and abnormal calcium handling. Furthermore, samples analysed by electron microscopy suggest that the mutant cardiomyocytes possess disorganised myofibrils. Any differences in mitochondrial structure or alterations to the cellular proteome of these cardiomyocytes await further validation.

Conclusion: The hESC mutants generated here have the potential to provide clinically relevant tissue samples for functional analyses of disease pathogenesis, and further investigation of the molecular mechanisms involved in mitochondrial protein biogenesis. Ultimately, these cells can be used to facilitate pre-clinical studies to test for potential treatments for Sengers syndrome.

OP20. DOES CIGARETTE SMOKING EXACERBATE HIGH-FAT DIET INDUCED HEPATOSTEATOSIS TO NASH IN C57BL/6J MICE

Sherouk Fouda[1], Anwar khan[1], Ali Mahzari[1], Xiu Zhou[1], Ross Vlahos[2], Ji-Ming Ye[1]

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2. *Chronic Infectious and Inflammatory Diseases Research Laboratory, School of Health and Biomedical Sciences, RMIT University, Melbourne, Australia*

Over consumption of dietary fat and cigarette smoking are some of the common concurrent risk factors implicated in the development of non-alcoholic steatohepatitis (NASH). Our previous studies in mice showed that high-fat diet (HFD) alone can cause hepatosteatosis as a hepatic manifestation of the metabolic syndrome but not sufficient to result in NASH. This study aimed to test the hypothesis that cigarette smoking may exacerbate HFD-induced hepatosteatosis to NASH by oxidative stress as an additional hit.

C57BL/6J mice were exposed to two cigarettes twice daily, 5 days/ week for 14 weeks along with HFD feeding. In standard diet fed mice, cigarette smoking did not show any significant effects on lipid content in the liver or other signs of NASH. As expected, HFD resulted in marked obesity, hypertriglyceridemia, glucose intolerance and hepatosteatosis but without other pathological characteristics of NASH. Interestingly, cigarette smoking sustained HFD-induced hypertriglyceridemia, glucose intolerance and hepatosteatosis even though the HFD-induced body weight gain and adiposity was significantly reduced. Furthermore, the HFD mice exposed to cigarette smoking displayed significant increases in the mRNA levels of TNF-α, IL-1β and α-SMA in the liver, indicating a progression towards NASH. Further studies are under way to examine the histological features of inflammation and fibrosis in the liver and the role of oxidative stress. Findings from study are expected to provide new insight into the pathogenesis of NASH initiated from these two lifestyle factors.

OP21. USING A GENE EXPRESSION SIGNATURE TO IDENTIFY NOVEL BETA-CELL PROTECTIVE COMPOUNDS

Bhavisha Patel[1], Smithamol Sithara[1], Megan Ellis[1], Tamsyn Crowley[2], Ken Walder[1] and Kathryn Aston-Mourney[1]

[1]*Centre for Molecular and Medical Research, School of Medicine, Deakin University, Geelong, Australia*

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Introduction: Type 2 Diabetes (T2D) is a chronic condition of high blood glucose levels due to inadequate amounts of the glucose-lowering hormone, insulin. The major contributor to T2D is dysfunction of the insulin-producing beta-cells. The dysfunction of these cells is progressive and results in worsening of the disease over time. Current drugs for T2D assist with the management of symptoms but do not protect or prevent further damage to beta-cells. Beta-cell dysfunction has complex pathology and is still incompletely understood. Previous drug discovery methods have focused on a single target which has proven to be ineffective due to the complexity of beta-cell dysfunction. An unbiased and unique approach of gene expression signatures (GES) has previously been used successfully in other complex diseases by providing a genetic fingerprint that is representative of the overall function and phenotype of the cell.

Aim: To develop a beta-cell GES to identify compounds that have a protective effect on beta-cells in a diabetic environment.

Methods: A model of beta-cell dysfunction and reversal of dysfunction was previously developed using INS-1E cells, a rat beta-cell line, and gene expression profiling conducted. Differentially expressed genes were validated using qPCR in two independent sets of samples. Genes that passed these two rounds of validation were used to generate our beta-cell GES. This GES was then used to screen a publically available database of compounds (Connectivity Map; Broad Institute) and the top eight compounds were tested *in vitro*. Cells were cultured with 10µM of each compound and insulin secretion and apoptosis (caspase-3/7 activity) were measured (n=6).

Results: Results were compared using two-way ANOVA with LSD post-hoc analysis with $p<0.05$ being considered significant. Of the eight compounds, five improved insulin secretion and two also reduced apoptosis.

Conclusion: Our GES was capable of identifying compounds that have a protective effect on beta-cells and may prove to be new T2D treatment options.

Poster Presentations

Judging Time

P2-P17

10:35-11:00

Pages 38-52

P18-P35

13:05-13:50

Pages 53-69

All poster presentations will be held in Brennan Hall

P-2. USING A GENE EXPRESSION SIGNATURE TO IDENTIFY NOVEL BETA-CELL PROTECTIVE COMPOUNDS

Bhavisha Patel[1], Smithamol Sithara[1], Megan Ellis[1], Tamsyn Crowley[2], Ken Walder[1] and Kathryn Aston-Mourney[1]

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Results: Results were compared using two-way ANOVA with LSD post-hoc analysis with $p<0.05$ being considered significant. Of the eight compounds, five improved insulin secretion and two also reduced apoptosis.

Conclusion: Our GES was capable of identifying compounds that have a protective effect on beta-cells and may prove to be new T2D treatment options.

P-3. SPEECH DISCRIMINATION AND COGNITIVE DYSFUNCTION IN MULTIPLE SCLEROSIS

Pippa Iva[1], Russell Martin[1], Joanne Fielding[2], Meaghan Clough[2], Owen White[2], Ramesh Rajan[3]

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Introduction: Our most complex interactions with fellow human beings occur through speech but often in unfavourable conditions such as in the presence of background noise or in reverberant conditions. In these conditions, listeners must use a number of sensory and cognitive resources to extract the speech of interest. Multiple Sclerosis (MS) pathology can cause disruptions in central auditory processing and higher-level cognitive control processes and these may impair the ability to discriminate speech in background noise despite normal hearing function.

Aims: To determine speech discrimination abilities in Relapsing-Remitting Multiple Sclerosis (RRMS) in several listening conditions with varying cognitive demand.

Methods: The hearing status of all participants was determined via standard audiology procedures to exclude any subjects with hearing loss. Pre-recorded Bamford-Kowal-Bench (BKB) sentences and keywords were presented via headphones at a constant level of 70 dB in two types of background noise: speech weighted noise and eight-talker babble. The volume of the background was manipulated to present at 6 different signal-to-noise ratios. Subjects completed a questionnaire that subjectively identified any speech discrimination difficulties in daily living listening scenarios. RRMS subjects also completed standard neuropsychological tests such as the California Verbal Learning Test, Symbol Digits Modalities Test and Paced Auditory Serial Addition Test.

Results: MS subjects had small, but significant impairments in dissociating sentences and words from multi-talker babble but not with speech-weighted steady noise; indicating greater MS related effects on informational as opposed to energetic masking. Despite this, RRMS subjects reported no difficulties on the three components of the questionnaire: audio-attentional difficulty and auditory discomfort for verbal and non-verbal stimuli. Several significant correlations were revealed between performances on our speech discrimination tasks and standardised neuropsychological measures of attention, working memory, inhibitory control and executive function.

Conclusions: These results identify novel deficits in high-level auditory processing in MS and show how these can be explained on the basis of standard tests of cognitive function. These correlations may provide the basis for a cost-effective and non-invasive evaluation of sensory and cognitive evaluation in MS, providing a marker of disease severity and progression for MS.

P-4. Metabolomic analysis uncovered the synergistic mechanisms of polymyxin B in combination with rifampicin against MDR *Acinetobacter baumannii*

Jinxin Zhao[1], Yu-wei Lin[1], Mei-Ling Han[1], Darren Creek[2], Tony Velkov[3], Yan Zhu[1], Jian Li[1]

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Background: Polymyxins are currently used as last-line therapy against multidrug-resistant (MDR) *Acinetobacter baumannii*. As resistance to polymyxins can emerge in *A. baumannii* with monotherapy, combination therapies are often employed in the clinic. Previous studies showed that polymyxin-rifampicin combination displayed synergistic killing against MDR *A. baumannii*; however, the synergistic mechanism remains unclear. In the present study, we employed metabolomics to investigate the synergistic mechanisms of polymyxin B-rifampicin combination against a model strain of MDR *A. baumannii* AB5075.

Methods: Bacterial log-phase culture was treated with polymyxin B (0.75 mg/L), rifampicin (1 mg/L), and their combination, respectively. Samples were collected at 0, 1, 4 and 24 hr, and LC-MS was employed to analyse the metabolome. MzMatch, IDEOM and MetaboAnalyst were used for bioinformatic analysis. Significant metabolites were defined as fold change > 2, FDR < 0.05.

Results: Polymyxin B monotherapy only caused early (1 hr) perturbation of Phosphatidylethanolamines metabolism (e.g. *sn*-glycero-3-phosphoethanolamine). Rifampicin monotherapy induced significant perturbations in nucleotide and amino acid metabolism (14 metabolites) at 4 hr. More key metabolic pathways (e.g. energy, lipid, nucleotide, amino acid metabolism) were significantly perturbed by the combination at 1 and 4 hr (36 and 61 significant metabolites, respectively). Significant changes in the levels of glycerophospholipids and fatty acids were observed after the combination treatment for 1 and 4 hr. Of particularly interested is that the combination exclusively increased the intermediate metabolite pools in pentose phosphate pathway at 1 hr. Furthermore, the pyrimidine metabolism and histidine degradation pathways were significantly increased. Interestingly, metabolites in the nucleotide and amino acid biosynthesis pathways were significantly decreased at 4 hr. Compared to each monotherapy, most key metabolic pathways were disrupted by this combination.

Conclusions: This is the first study to employ metabolomics to unveil the synergistic killing mechanisms by polymyxin-rifampicin combination against MDR *A. baumannii*. The time-dependent synergistic activity via disruption of PPP, nucleotide and amino acid metabolism will help design better polymyxin combinations in the clinic.

P-5. INVESTIGATING THE TUMOUR SUPPRESSOR FUNCTIONS OF P53

Elizabeth Lieschke[1,2], Gemma Kelly[1,2], Andreas Strasser[1,2].

[1] The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, [2] Department of Medical Biology, University of Melbourne, Melbourne, Victoria

Introduction: Appropriate expression and function of the transcription factor p53 is required for effective tumour suppression and the prevention of cancer. This is evident by the high mutational rate of p53 (~50%) found across the spectrum of human cancers. p53 is activated by diverse cellular stresses, including activation of oncogenes, accumulation of reactive oxygen species and DNA damage. Once activated, p53 can initiate a range of tumour suppressive cellular responses, such as apoptotic cell death, cell cycle arrest, cell cycle senescence and various DNA repair processes. However, the factors that determine which one of these many p53-induced processes is activated in a particular cell are unknown. In our experiments we use the MDM2 inhibitor, nutlin-3a, that can specifically activate p53 in a non-genotoxic manner.

Aims: To characterise the response of a range of wild-type (wt) p53 containing human tumour cell lines of different cell origins to the non-genotoxic p53 activator, nutlin-3a, and to determine the cellular factors which govern the nature of the response.

Methods: Tumour derived cell lines were confirmed to express wt p53 using a next-generation sequencing assay, and assessment of p53 protein levels pre- and post- p53 activation using nutlin-3a by Western blotting. Cell cycling was assessed by flow cytometry using DAPI staining for DNA content and apoptotic cell death was measured by flow cytometry using staining with Annexin-V plus PI. Cellular senescence was assessed using staining for senescence associated beta-galactosidase.

Results: Various human tumour cell lines of epithelial (SJSA-1, U87MG, A172) or haematological origin (DoHH2, Sudhl-5) were confirmed to express wt p53. The response to p53 activation by nutlin-3a, was tested at 24 and 72 h. Some cell lines (DoHH2, SJSA-1) underwent apoptosis and a dose dependent response to nutlin-3a treatment was observed. By contrast, other cell lines (Sudhl-5, U87MG, A172) did not die and instead underwent cell cycle arrest and/or senescence.

Conclusions: We have found that human tumour-derived cell lines undergo one main p53-mediated cellular response upon nutlin-3a treatment in a dose dependent manner. This research will be the foundation to further investigate and determine what causes cells to commit to different responses following p53 activation.

P-6. EXPLORING REGRESSION TO THE MEAN IN AN AUSTRALIAN COHORT OF YOUNG HEAVY DRINKERS

JUN-TING YEUNG[1,2], CASSANDRA JC WRIGHT[1,3], MICHAEL LIVINGSTON[4], SARAH CALLINAN[4], PAUL DIETZE[1,3]

[1]Burnet Institute, Melbourne, Victoria, Australia [2]University of Melbourne, Melbourne, Victoria, Australia, [3]Monash University, Melbourne, Victoria, Australia [4]Centre for Alcohol Policy Research, La Trobe University, Melbourne, Victoria, Australia

Introduction: Alcohol consumption studies typically report average consumption generated through graduated-frequency measures or equivalent that are prone to regression to the mean (RTM), a statistical artefact whereby observations of a group approximate to the mean of that group. Individuals who are below the mean will increase, whereas individuals above the mean will decrease over time. Recently, studies have utilised peak consumption, another measure that estimates the maximum number of drinks consumed in a recent heavy drinking occasion.

Aims: Little is known about the stability of these measures, particularly amongst heavy drinkers. In this study we examined whether peak consumption is also subject to RTM, and whether other factors influence the stability of these measures over time.

Methods: Data were drawn from three waves (2012, 2013, 2015) of the Young Adults and Alcohol Study, a prospective cohort study of young Australian heavy drinkers (*n = 186*). Weekly consumption was derived from a graduated quantity-frequency measure. Peak consumption was estimated from self-reporting of a single heavy drinking occasion in the previous twelve months. The average amounts (in Australian Standard Drinks; ASD) of each method in each wave were compared. Analyses were carried out separately for gender and consumption tercile.

Results: We find an effect of RTM in weekly consumption. An increase in consumption was observed in the lower tercile (2012: 5.0ASD, 2013: 6.6ASD 2015: 7.0ASD) compared to a decrease in the higher tercile (2012: 31.1ASD, 2013: 20.74ASD 2015: 18.3ASD). Drinkers in the medium tercile stayed relatively consistent (2012: 12.7ASD, 2013: 11.0ASD, 2015: 13.7ASD). There was little evidence of RTM in peak consumption, instead a general increase was observed for both gender and terciles. Closer analysis revealed an effect of age. The youngest age group (18-21) displayed a general increase in consumption, whereas the older age group (22-25) remained relatively consistent. Additionally, average peak consumption was greater for the younger age group than the older age group across all waves.

Conclusions: Our findings demonstrate that there is a clear RTM-like pattern in weekly drinking. Preliminary examination suggests that peak consumption may be less susceptible to RTM than weekly consumption amongst heavy drinkers, suggesting its utility in measuring alcohol consumption trajectories. These results have implications for assessing the effects of interventions over time and studying alcohol consumption longitudinally.

P-7. THE ROLE OF PHOSPHORYLATION IN THE LOCALISATION AND PRESYNAPTIC FUNCTION OF ALPHA-SYNUCLEIN

Elysa Carr[1], Holly Melland[1], Mark Graham[2], Sarah Gordon[1].

[1] *The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC.*

[2] *Children's Medical Research Institute, Sydney, NSW.*

Introduction: The tightly controlled release of neurotransmitters from presynaptic nerve terminals is essential for neural network signalling. Repeated rounds of exocytosis and endocytosis of neurotransmitter-containing synaptic vesicles play a critical role in the maintenance of neurotransmission. The protein alpha-synuclein is a known modulator of the synaptic vesicle cycle, most likely via interactions with the essential synaptic vesicle protein synaptobrevin-II. Alpha-synuclein has long been implicated in the pathogenesis of Parkinson's disease, however its normal function has not yet been established. Novel phosphorylation sites on alpha-synuclein have recently been identified that may give clues to its physiological function at nerve terminals.

Aims: This study aimed to determine how novel phosphorylation sites of alpha-synuclein affect its localisation and function through the use of mutations that mimic or abolish phosphorylation at these residues.

Methods: Primary hippocampal neuron cultures were transfected with phosphomutant variants of alpha-synuclein generated through site-directed mutagenesis. Cells were either fixed at rest, after stimulation, or after a recovery period following stimulation. Neurons were immunolabelled and imaged with an epifluorescence microscope. Synaptic protein expression and localisation analyses were performed on alpha-synuclein and other presynaptic proteins both at rest and following neuronal activity.

Results: The synaptic localisation of most alpha-synuclein mutants is comparable to wild-type alpha-synuclein. However, its binding partner synaptobrevin-II is more diffuse along neurites in neurons expressing alpha-synuclein mutants that either mimic phosphorylation at the novel phosphorylation sites, or that are known to disrupt alpha-synuclein membrane-binding.

Conclusions: Phosphorylation of alpha-synuclein at novel residues may control the dissociation of alpha-synuclein from vesicular and presynaptic membranes, thereby impacting its ability to act on synaptobrevin-II. This furthers our understanding of the complex regulation of alpha-synuclein function at nerve terminals.

P-8. THE APPLICATION OF IMMUNOHISTOCHEMISTRY IN DIAGNOSIS OF RARE RENAL TUMOURS

An-Khuong Huynh[1], Andrew Strmecki[1], Ching-Tou Lin[1] & Zongyue Wang[1]

[1]*School of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria*

Introduction: The kidneys are vital organs that play a role in homeostasis by filtering blood and secreting hormones. Kidney cancer is one of the top ten most common cancers worldwide. Renal cell carcinoma (RCC) is the most common form of kidney malignancy accounting for about 90% of cases. The remaining 10% are rare types of kidney cancer that need to be differentiated from RCC. Nephroblastoma or Wilms tumour (WT) is rarely seen in adults, however, it is the most common type of renal cancer in children. The disease typically presents as a triphasic tumour with blastemal, epithelial and stromal components. Leiomyosarcoma is another rare malignancy that accounts for 0.5-1% of all renal cancers. The disease has poor prognosis and needs to be distinguished from sarcomatoid renal cell carcinoma as they share many morphologic characteristics. In the past, morphology was the only tool for diagnosis. Nowadays, immunohistochemistry (IHC) technique provides a specific and accurate mean for diagnosing these tumours.

Aim This study aimed to confirm the presumptive diagnoses of a WT and a leiomyosarcoma using special stains and IHC staining.

Methods: Archival paraffin blocks of a WT case and a leiomyosarcoma case diagnosed in 1955 were re-processed and re-embedded to ensure that they met today's technical standards. H&E and special stains were performed for morphological examination. IHC using antibodies against Wilms tumour 1 protein (WT1), keratins (AE1/AE3, CK7), vimentin, desmin and S100 was carried on the WT while smooth muscle actin (SMA), smooth muscle myosin heavy chain (SMMS), vimentin, desmin and Ki67 antibodies were used for the leiomyosarcoma.

Results: The WT case presented a typical triphasic pattern with blastemal, epithelial and stromal elements in H&E and Masson Trichrome staining. These components showed different staining results with WT1, AE1/AE3, CK7, vimentin, desmin and S100 antibodies. The leiomyosarcoma morphology displayed a characteristic pattern of spindle cells with cigar-shaped nuclei. The tumour also had positive staining for SMA, SMMS, vimentin, desmin and Ki67.

Conclusion: IHC staining technique was very useful to confirm the diagnoses of Wilms tumour and leiomyosarcoma of the kidney, especially in retrospective study of paraffin-embedded blocks from the past.

P-9. THE HUMAN ENDOMETRIAL EPITHELIAL RECEPTOME: DEFINING AND EVALUATING NOVEL PROTEIN BIOMARKERS OF ENDOMETRIAL RECEPTIVITY

Jennifer Hutchison[1,2], David W. Greening[3], Lois A. Salamonsen[1,2], Jemma Evans[1,2]

[1]Hudson Institute of Medical Research, Clayton, VIC; [2]Department of Molecular and Translational Science, Monash University, Clayton, VIC; [3]Department of Biochemistry and Genetics, Latrobe University, Melbourne, VIC.

Background: Endometrial receptivity is critical for successful reproduction: the endometrium must be receptive, and the blastocyst hatched and appropriately developed for adhesion and subsequent implantation. Although fertility clinics have optimised quality embryo production, success rates have not improved; The Australia and New Zealand Assisted Reproduction Database indicates little change in live birth rate (2010 – 2015: 18.1%). It is necessary to gain greater understanding of endometrial receptivity, and the factors involved, to provide targets for improving reproductive outcomes.

Aim: Determine and validate a proteomic signature of human endometrial epithelial receptivity (the receptome) and assess the functional role of individual proteins.

Methods: Hormonally-primed (estrogen/progesterone) primary human endometrial epithelial cells (HEEC) were co-cultured with spheroids of human trophoblast stem cells (TS) to mimic blastocyst adhesion to the endometrium. HEEC monolayers were designated 'adhesive' or 'non-adhesive' based on TS adhesion after 6-hours co-culture. Matched hormonally primed HEEC monolayer-only cultures were accordingly designated 'receptive' or 'non-receptive' and subjected to comparative proteomics. The differentially identified proteins defined the endometrial epithelial "receptome". Gene ontology analysis determined functional processes regulated by these proteins. Immunohistochemistry validated and localised individual proteins in 'receptive' (mid-secretory phase) and 'non-receptive' (proliferative phase) human endometrium. Comparisons were performed between fertile and primary infertile women. siRNA knockdown of specific receptome proteins in ECC-1 cells, coupled with TS adhesion, evaluated the functional significance of specific receptome proteins.

Results: Within the human endometrial epithelial receptome, 136 proteins were upregulated and 132 downregulated in 'receptive' versus 'non-receptive' HEEC monolayers. Highly upregulated proteins include CDA, LGMN, MAGT1, STMN1, and KYNU. CDA and STMN1 were previously identified in receptive endometrium, confirming the validity of this model. Gene ontology terms enriched in the upregulated receptome proteins include cellular protein complex disassembly, translation, neutrophil degranulation, and associated immune processes. Upregulated receptome proteins were localised in human endometrium and elevated in receptive versus non-receptive tissues. In comparison to fertile women, CDA and LGMN were reduced in the early secretory endometrium of primary infertile women. Following siRNA knockdown of STMN1 and LGMN, reduction in TS adhesion indicates that at least these receptome proteins are functionally significant in maintaining endometrial receptivity.

Conclusion: A human endometrial epithelial "receptome" has been defined and validated, providing a novel protein fingerprint of endometrial receptivity. Specific identified proteins are important for embryo adhesion to the endometrium. Furthermore, receptome protein expression is altered in women with primary infertility. Receptome characterization provides targets for improving endometrial receptivity or novel non-hormonal contraceptives.

P-10. EFFICIENT AND AUTOMATED NEURONAL TRACKING ON GLOBAL BRAIN IMAGING WITH POINT REGISTRATION

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Introduction: Global Brain Imaging allows investigation of neural dynamics on large numbers of neuron at a whole ganglion level in addition to single neuron activity. Caenorhabditis elegans is a powerful model organism as it is the only animal model whose nervous system structure has been completely mapped, presenting a unique opportunity for complete mapping of the relationship between neural structure and function. However, the bottleneck of large scale data analysis requires extensive manual corrections both time- and labor-intensive, motivating this project to develop a point registration algorithm for efficient and automated neuronal tracking.

Aim: To apply an automated algorithm on a larger sampling size, thereby achieving efficiency at both the data acquisition and analysis level for whole brain neuronal tracking, thus proposing a more comprehensive insight into neural dynamics.

Methods: Each Global Brain Imaging video contains multiple frames where each frame denotes a specific time point. The algorithm uses a point set registration algorithm to track neurons followed by Principal Component Pursuit to mitigate sparse noise. To validate the proposed algorithm, we compare our data with manual annotations data from previous studies on the same videos. We also conducted additional whole-brain imaging recordings, using microfluidic systems for high-throughput data acquisition. Changes in the neuronally-expressed Ca2+ sensor GCaMP is monitored through fluorescence, a marker of neural activity. The data gathered from the larger population of worms is then used to obtain a window into the firing behaviour of head neurons, suggesting further investigation into the dynamic coordination of neural circuitry.

Results: The developed tracking algorithm validates previous studies by showing the same fluorescent traces on the same neurons. By matching points representing neurons of a single frame to the reference frame, the proposed algorithm tracks neurons with high accuracy and efficiency. To expand insight into neural dynamics on a larger sampling size, additional worms were imaged via microfluidic devices, enabling efficiency both at the data acquisition and data analysis level.

Conclusion: Automated neuronal tracking through point registration can be used for global brain imaging analysis, achieving comparable accuracy with higher efficiency. The neuronal tracker suggests profitable implications in neuroscience research and further investigation on neural dynamics and circuitry.

P-12. POSTNATAL DEVELOPMENTAL TRAJECTORY OF DOPAMINE RECEPTOR 1 AND 2 EXPRESSION IN CORTICAL AND STRIATAL BRAIN REGIONS.

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Introduction: Adolescence is a unique developmental period during which numerous biological and physiological changes occur. It is a period of heightened vulnerability for developing mental disorders, including substance use, schizophrenia, and anxiety disorders. Although the biological bases for this vulnerability are uncertain, they could be related to an imbalance in dopamine receptor levels in the adolescent brain compared to the adult brain. The precise postnatal developmental trajectory of these receptors is poorly understood, especially at key cognitive milestones. We hypothesised that adolescents express more cells with dopamine receptor mRNA in discrete neural regions compared to adults.

Aim: To investigate the postnatal developmental trajectory of D1 and D2 expressing neurons in the prelimbic cortex (PL) and infralimbic cortex (IL) of the mPFC, insula cortex, and dorsal and ventral striatum of female and male mice.

Methods: We used stereological software to investigate the development of dopamine receptor 1 (D1) and 2 (D2) gene-expressing neurons in the prelimbic and infralimbic cortices of the medial prefrontal cortex, insula cortex, dorsal striatum, and ventral striatum at postnatal day (P) 17 (juvenile), P25 (preadolescent), P35 (early adolescent), P49 (late adolescent) and P70 (adult), using transgenic mice expressing green fluorescent protein under the control of the D1 or D2 promoter. We estimated the total number and density of D1 and D2 positive neurons in these regions, as well as the volume of these regions.

Results: D1:D2 density was the most erratic in the insula cortex. Specifically, there was a substantial increase from late-adolescence into adulthood in males but not in females. D1:D2 density in the prelimbic and infralimbic cortices were not affected by age, and in the dorsal and ventral striatum it increased across maturation. Additionally, D1:D2 density in all regions examined was higher in females compared to males.

Conclusions: Taken together, these results suggest that late-adolescent males may be more vulnerable to developmental disorders compared to adult males due to their lower D1:D2 density ratio in the insula cortex compared to adolescent females and adult males. That is, we have identified the insula cortex as a novel locus in which enhanced expression of D1 compared to D2 may be protective against certain developmental disorders.

P-13. POLYMER THERAPEUTICS – TARGETED DRUG DELIVERY FOR METASTATIC CANCER

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Introduction: One in two Australian will be diagnosed with cancer by the age of 85. Unfortunately, 32% of the cancer patients will die within five years of the diagnosis. The predominant cause of this high death rate is metastasis, the process of cancer cells migrating and re-establishing multiple secondary tumours that are unable to predict, prevent, manage, and treat. Target-oriented nanomedicine has shown some success in tumour delivery in past decades without much of emphasis on metastatic cancer.

Aims: We aim to develop a target-oriented, polymer-based delivery system for metastatic cancer and quantify the amount of the nanomedicine inside metastatic cancer cells *in vivo*.

Method: We are focusing on delivery to metastatic melanoma in this project, and the system is applicable for another cancer type by changing the target agents. We start with designing and synthesising the polymer backbone for desire molecular weight, functionality, and surface chemistry using Reversible Addition-Fragmentation Chain-transfer Polymerization. Afterwards, we add the targeting agents [Nle^4, $D\text{-}Phe^7$]-α-Melanocyte-stimulating hormone, a potent peptide that highly selective to melanocortin 1 receptor (MC1R), to the polymer backbone which provides specificity to the melanoma cells with the overexpressed receptors. With the prototype, we evaluate its specificity by peptide activity evaluation *in vitro* and biodistribution of the system *in vivo* using the syngeneic B16 melanoma mouse model.

Results: After screening a series of copolymers, we chose a slightly negative charged copolymer to consist with N-acryloylmorpholine (NAM) and acrylic acid (AA) with around 25,000$gmol^{-1}$ molecular weight and 6nm diameter as the polymer backbone based on the properties of human serum albumin. We conjugated the peptide on to the polymer using pentafluorophenyl acrylate and later remove the rest of pentafluorophenol group to leave acrylic acid on the polymer at the end. We evaluated the activity of the conjugated peptide by comparing with the free peptides. We determined the effect of the peptide-receptor binding by measuring the amount of cyclic adenosine monophosphate (cAMP) secreted by the cells from the receptor stimulation. Despite the expected decrease in the peptide activity, the peptide is still active enough for receptor binding.

Conclusion: From the result of peptide activity, we determine the prototype has enough specificity to the cancer cells for targeted delivery. We continue the project by evaluating the internalisation of the prototype *in vitro* and following with *in vivo* study next.

P-14. DOES CIGARETTE SMOKING EXACERBATE HIGH-FAT DIET INDUCED HEPATOSTEATOSIS TO NASH IN C57BL/6J MICE

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Over consumption of dietary fat and cigarette smoking are some of the common concurrent risk factors implicated in the development of non-alcoholic steatohepatitis (NASH). Our previous studies in mice showed that high-fat diet (HFD) alone can cause hepatosteatosis as a hepatic manifestation of the metabolic syndrome but not sufficient to result in NASH. This study aimed to test the hypothesis that cigarette smoking may exacerbate HFD-induced hepatosteatosis to NASH by oxidative stress as an additional hit.

C57BL/6J mice were exposed to two cigarettes twice daily, 5 days/ week for 14 weeks along with HFD feeding. In standard diet fed mice, cigarette smoking did not show any significant effects on lipid content in the liver or other signs of NASH. As expected, HFD resulted in marked obesity, hypertriglyceridemia, glucose intolerance and hepatosteatosis but without other pathological characteristics of NASH. Interestingly, cigarette smoking sustained HFD-induced hypertriglyceridemia, glucose intolerance and hepatosteatosis even though the HFD-induced body weight gain and adiposity was significantly reduced. Furthermore, the HFD mice exposed to cigarette smoking displayed significant increases in the mRNA levels of TNF-α, IL-1β and α-SMA in the liver, indicating a progression towards NASH. Further studies are under way to examine the histological features of inflammation and fibrosis in the liver and the role of oxidative stress. Findings from study are expected to provide new insight into the pathogenesis of NASH initiated from these two lifestyle factors.

P-15. MONOUBIQUITINATION LOCKS FANCI:FANCD2 ON DNA

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Introduction: The Fanconi anemia (FA) pathway is required for efficient repair of interstrand crosslink (ICL) DNA damage. In the majority of FA patients, FANCI:FANCD2 monoubiquitination is absent, highlighting the importance of monoubiquitination in repairing ICL. Currently it remains unclear how monoubiquitination of FANCI:FANCD2 activates a downstream ICL repair pathway.

Aim: This monoubiquitination reaction is defective in 95% of FA patients so we sought to better understand how this process is regulated.

Methods: To do so, we have developed a novel biochemical system that reconstitutes the FA pathway *in vitro* with human proteins. This allowed us to study protein:DNA interaction using fluorescentlly labelled DNA containing different DNA structures.

Results: Using a reconstituted *in vitro* ubiquitination assay, we show that monoubiquitination of FANCI:FANCD2 is robustly stimulated by different DNA structures. When the affinity of monoubiquitinated and unmodified (apo)-FANCI:FANCD2 complex was compared using electromobility shift assay (EMSA), we observed monoubiquitinated FANCI:FANCD2 complex stabilized on DNA. We also confirmed that the gel shifted band contains both FANCI and FANCD2 proteins, suggesting that the proteins act as a dimeric complex during DNA interactions. Addition of competitor DNA *after* FANCI:FANCD2 was monoubiquitinated prevented it from being displaced, but addition *before* monoubiquitination caused a concentration-dependent inhibition of EMSA shift. This result can be explained by a model where apo-FANCI:FANCD2 moves freely between the unlabelled and labelled DNA molecules, but after monoubiquitination, it is immobilized on the labelled DNA.

Finally, we show that the reversal of the ubiquitination reaction by the USP1:UAF1 deubiquitinase unlocks FANCI:FANCD2 from the DNA. It is then free to move to new substrates. This result suggests that USP1:UAF1 plays a critical role in the transport of FANCI:FANCD2 on damaged DNA, and the prevention of abnormal activation of the FA pathway.

Conclusions: Together, our results demonstrate that monoubiquitination of FANCI:FANCD2 complex "locks" it on DNA. We are currently exploring how the locking mechanism controls DNA repair, either through direct stabilization of DNA repair intermediates and/or the recruitment of additional factors.

Significance: These results demonstrate the mechanistic basis for temporal and spatial control of the FANCD2:FANCI monoubiquitination that is critical for chemotherapy responses and prevention of Fanconi anaemia.

P-16. The role of geospatial hotspots in the spatial spread of tuberculosis in rural Ethiopia: A mathematical model

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Introduction: Geospatial tuberculosis hotspots are hubs of TB transmission both within and across community groups.

Aims: We aimed to quantify the extent to which these hotspots account for the spatial spread of TB in a high-burden setting.

Methods: We developed spatially coupled models to quantify the spread of TB from geographic hotspots to distant regions in rural Ethiopia. The population was divided into three 'patches' based on their proximity to transmission hotspots, namely hotspots, adjacent regions and remote regions. The models were fitted to five-year notification data aggregated by the metapopulation structure. Model fitting was achieved with a Metropolis-Hastings algorithm using a Poisson likelihood to compare model-estimated notification rate with observed notification rates.

Results: A cross-coupled metapopulation model with assortative mixing by region closely fit to notification data as assessed by the Deviance Information Criterion. We estimated 45 hotspot-to-adjacent regions transmission events and 2 hotspot-to-remote regions transmission events occurred for every 1000 hotspot-to-hotspot transmission events. Although the degree of spatial coupling was weak, the proportion of infections in the adjacent region that resulted from mixing with hotspots was high due to the high prevalence of TB cases in a hotspot region, with approximately 75% of infections attributable to hotspot contact.

Conclusions: Our results suggest that the role of hotspots in the geospatial spread of TB in rural Ethiopia is limited, implying that TB transmission is primarily locally driven.

P-17. THE USE OF ADVANCED DIAGNOSTICS ON ARCHIVAL TISSUE FOR THE DIAGNOSIS OF RARE DISEASES IN THE FEMALE REPRODUCTIVE SYSTEM

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Introduction: In 2014, ovarian cancer was identified as the eighth most common cancer affecting women in Australia. The incidence of ovarian cancer is higher in women aged between 50-70 years of age, with approximately 75% of cases diagnosed in women over the age of 55. Early detection of ovarian tumours is crucial for patient prognosis and management; however the likelihood of early diagnosis is rare due to late presenting symptoms. According to the World Health Organisation, ovarian tumours can be classified into three major categories including, epithelial tumours, sex cord-stromal tumours, and germ cell tumours. Epithelial tumours are the most commonly diagnosed ovarian tumour subtype, accounting for up to 95% of all ovarian tumours. Sex cord-stromal tumours are considered rare, accounting for 1.2% of all ovarian cancers. Immunohistochemistry (IHC) is a useful technique for the differentiation of ovarian subtypes as it allows for visualisation of specific components that cannot be revealed by haematoxylin and eosin (H&E) staining and special stains.

Aim: To use advanced diagnostics to confirm the presumptive diagnosis of archival tissues dating back to the 1950's.

Methods: The study was conducted using 10 rare archival paraffin-embedded tissues of the female reproductive system. The pre-analytical aspects of the tissue were unknown, as blocks were from 1955-1980. Tissues were re-embedded from original wooden moulds to fit the modern day microtome. An haematoxylin and eosin (H&E) stain was used to initially evaluate tissues to identify key structures essential for diagnosis. Based on additional research and the H&E findings, special stains and IHC were used to confirm the presumptive diagnoses.

Results: It was found that IHC is useful in diagnosing ovarian tumour subtypes, specifically endometroid carcinomas and granulosa-theca cell tumours. Calretinin, vimentin and EMA were most useful for differentiation. Some tissues displayed weak staining; this could be due to completing IHC on archival tissues.

Conclusions: Throughout this study, IHC has proven to be useful in the accurate diagnosis of ovarian tumours; however it was found that this technique is not essential in all diseases of the female reproductive system. For cases such as ovarian pregnancies and cysts, a diagnosis can be determined using only H&E staining and other special stains.

P-18. IDENTIFYING EPISTASIS UNDERLYING AGE-RELATED MACULAR DEGENERATION (AMD) TO UNDERSTAND THE GENETIC ARCHITECTURE OF THE DISEASE.

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Background: Age-related Macular Degeneration (AMD) is a disease of macula, leading to the loss of central vision affecting the elderly population of developed countries. AMD is a complex disease caused by multiple genetic and environmental factors. The 52 variants discovered by the IAMDGC (International AMD Gene Consortium) in the 34 loci associated to AMD so far explain over 50% of genetic variance and 27.2% of total disease variance. The missing variance of AMD may be explained from several sources and the role of gene-gene (GxG) interactions may be one such factor.

Methods: To identify the GxG, three different tools based on three different statistical techniques namely PLINK based on logistic regression, BOOST [3] (Boolean Operation based Screening and Testing) based on logistic regression and log-linear models and Antepiseeker [5] based on ant colony optimisation techniques are used. Consistency of interactions across 7 cohorts of IAMDGC is being taken as one of the criterion to investigate the efficiency of the tools.

Results: Using PLINK, we identified two intronic single nucleotide polymorphisms (SNPs) (rs6695321 & rs424535) from the CFH gene showed an additive effect and replicated across 6 of 7 cohorts with a combined Fisher's p-value of 1x10-46 (OR=1.8-2.4). Seven other CFH-CFH interactions were observed across 5 cohorts. Using BOOST, 10 interactions were identified across 5 cohorts and 12 interactions consistent across 4 cohorts with the top interaction being between seq-rs7513157 and kgp15357111(p=1X10-39). Using Antepiseeker there was only one interaction between rs7540032 and rs3750847 consistent across two cohorts.

Conclusion: In this preliminary analysis on cross-Cohort consistency, PLINK showed the highest while Antepiseeker least consistency. Variability between epistatic techniques appears to be a limitation in this field.

P-19. **THE USE OF IMMUNOHISTOCHEMICAL AND SPECIAL HISTOLOGICAL STAINING TECHNIQUES TO CONFIRM TERATOMA DIAGNOSIS.**

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Teratomas are a type of pure germline cell tumour which can be categorized as mature or immature. The tumours develop from defective pluripotent germline cells and are composed of one or more of the three germ layers. The three germ layers consist of the endoderm which differentiates to mucinous and ciliated epithelium, the mesoderm which differentiates to skeletal, muscular and connective tissue systems and the ectoderm which differentiates to hair, skin, nails and teeth. A mature teratoma is defined by well-differentiated internal structures and are most often benign. An immature teratoma is defined by poorly differentiated internal structures and often demonstrate malignant behaviour. Historically, teratomas were diagnosed through imaging techniques or routine haematoxylin and eosin (H&E) staining and more recently immunohistochemical techniques to assist with differential diagnosis. We have used advanced histopathological laboratory techniques to confirm historical diagnosis of teratomas through the use of immunohistochemistry (IHC) and special staining techniques.

Through the examination of the H&E sections we were able to deduce relevant IHC and special staining techniques to support or disprove previous diagnosis. Seven previously diagnosed teratoma cases were microscopically examined and special stain techniques were performed such as Masson trichrome to assess tissue structure and Periodic Acid Schiff (PAS), Alcian Blue PAS and PAS Diastase to assess glandular structures seen throughout all cases. IHC was also completed with antibodies such as Placental Alkaline Phosphatase (PLAP) which assists differentiating mat0r00ure from immature teratomas and using the cytokeratin (CKAE 1 /3) as confirmation. Of the seven cases, five presented as immature teratomas due to a negative PLAP staining pattern, one of which showing malignant transformation; two stained positive suggesting a mature teratoma. These results were confirmed through the use of CKAE 1 /3 which shows positive staining for mature teratomas only. The use of special staining techniques was used to assist with identifying tissue structures but not for diagnosis. Advanced histological techniques allowed for confirmation of previous diagnosis of presented cases, the use of IHC and special stain techniques had diagnostic value. However, IHC assisted with tumour categorization and therefore could better inform downstream treatment options.

P-20. c-Myc and Metabolic Reprogramming in Liver Cancer

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer, originating from the hepatocytes. Many cases of HCC involve the amplification of the oncogene c-Myc. c-Myc is a transcription factor that plays a key role in many aspects of cancer including cellular growth and metabolism. Metabolic reprogramming has recently emerged as a fundamental hallmark of cancer. Interestingly, recent studies have shown that c-Myc can regulate expression of genes associated with the *de novo* nucleotide biosynthesis and lipogenesis. Based on these studies, we hypothesize that c-Myc reprograms nucleotide and lipid metabolism to fuel liver cancer. In this project, we took advantage of an inducible zebrafish model of liver cancer, where c-Myc is overexpressed specifically in the hepatocytes upon exposure to doxycycline (TO-myc). Using this model, we demonstrated that c-Myc induces hepatomegaly in larvae and liver cancer in adults. In order to examine the impact of c-Myc on metabolism, we applied a metabolomics profiling strategy on dissected liver tissue, and we found that c-Myc overexpression reprogrammed metabolism. Based on these findings, we used TO-myc zebrafish as a drug discovery platform to screen potential metabolic interventions for efficacy in suppressing c-Myc driven hepatomegaly. Strikingly, we identified Mycophenolic acid, Simvastatin, Ezetimibe, and Orlistat as the compounds with the most profound effects in suppressing hepatomegaly driven by c-Myc. Together, our studies demonstrate the important role that metabolism plays in c-Myc driven liver growth and cancer. Furthermore, this work highlights the potential of using zebrafish models of liver cancer to identify therapeutic strategies that target the metabolic vulnerabilities of liver tumours.

P-21. HISTOPATHOLOGICAL EXAMINATION OF HYDATID CYSTS

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This study was to confirm the original diagnoses of hydatid cysts from two histopathological cases made by a retired pathologist between 1955 & 1980 using modern staining techniques.

Human hydatid disease, usually known as hydatid cyst, is caused by an infection of tapeworms of the genus *Echinococcus.* Although there are four species of genus *Echinococcus*, *Echinococcus granulosus* (*E.granulosus*) and *Echinococcus multilocularis* (*E.multilocularis*) are widely known to be principle causes of hydatid cysts in the liver and lung respectively. In most cases, hydatid cysts consist of three layers: adventitial layer containing collagen and host cells; laminated layer comprising of polysaccharide proteins; and germinal layer containing glycogen granules. Hydatid cysts have been reported in different areas of the world particularly in Africa, Latin America and Southeast Asia. Humans are infected with *Echinococcus* by the digestion of contaminated food and close contact with infected cattle. Haematoxylin & eosin (H&E) and special stains are primary diagnostic techniques used to confirm hydatid cysts in this study.

After paraffin blocks were sectioned at 4µm with the microtome, the sections were stained with Periodic Acid Schiff (PAS) +/- diastase, Masson's trichrome (MT) and Grocott's methenamine silver (GMS) for the confirmation of distinctive cystic structures detected in the H&E stain.

The protoscolex (also known as tapeworm's eggs) and structures of the cyst such as laminated and adventitial layers were well demonstrated with PAS, GMS and MT stains. In addition, some abnormal structures of the liver particularly fibrosis and cirrhosis were also well demonstrated with MT stain.

In conclusion, H&E and special stains are the main histopathological tools used to confirm the original diagnosis of hydatid cysts made by the pathologist between 1955 & 1980. The distinctive structures of the cyst and some pathological conditions of the liver were well demonstrated with H&E and special stains.

P-22. LEPTIN RECEPTOR MEDIATES CORTICAL BONE FORMATION

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Cortical bone is the thick outer shell of long bones, and is responsible for bone strength. Most fractures occur through cortical bone, but there is no targeted therapy that can improve cortical strength. This might be achieved by improving cortical bone formation (corticalisation). Corticalisation occurs during bone growth at the metaphysis, when newly formed trabecular bone coalesces at the bone periphery, and progressively increases in mineral density. However, it is not known how this process happens nor which signalling pathways are involved. In previous work, we knocked down the STAT3 inhibitor SOCS3 in osteoblasts and osteocytes *in vivo* using a Dmp1Cre transgene. This led to increased STAT3 phosphorylation in osteocytes, and delayed metaphyseal cortical bone formation; this could be seen clearly in 12w old females. This indicated that corticalisation requires SOCS3 inhibition of cytokine activity. One SOCS3-dependent cytokine receptor expressed in osteoblasts/osteocytes is the Leptin receptor (LepR). In order to determine whether hyperactive local LepR signalling was the cause of delayed corticalisation in *Dmp1Cre.Socs3*$^{f/f}$ mice, we generated double knockdown *Dmp1Cre.Socs3*$^{f/f}$*.LepR*$^{f/f}$ mice. This did not correct the delay in corticalisation. In contrast, using a micro-CT analysis on 12 week old *Dmp1Cre.Socs3*$^{f/f}$*.LepR*$^{f/f}$ female mice, we observed a further delay in corticalisation; cortical bone porosity was 50% lower than sex and age matched *Dmp1Cre.Socs3*$^{f/f}$. This indicates that LepR promotes the process of proper cortical bone formation. Furthermore, using three different thresholds to identify areas of low, mid and high density bone, we observed that accrual of high density bone was also suppressed by LepR deletion: female *Dmp1Cre.Socs3*$^{f/f}$*.LepR*$^{f/f}$ had 50% less high density bone than female *Dmp1Cre.Socs*$^{f/f}$, and 25% less mid density bone. Instead, they had 25% more low density bone than female *Dmp1Cre.Socs*$^{f/f}$. By histology, female *Dmp1Cre.Socs3*$^{f/f}$*.LepR*$^{f/f}$ mice had more osteoblasts and more active bone formation than controls, and abnormal, fibrotic bone marrow in the metaphysis. This data indicates that the delayed corticalisation of 12w old female *Dmp1Cre.Socs3*$^{f/f}$ mice is worsened by LepR deletion. This implies that LepR mediates the consolidation of cortical bone and the increase in mineral density that occurs during cortical bone formation.

P-23. IDENTIFYING *PLASMODIUM VIVAX* ENCODED PROTEINS THAT PREVENT HOST CELL DEATH DURING LIVER DEVELOPMENT

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Introduction and Aims: Malaria is a major global health problem and a leading cause of deaths worldwide. The mechanism behind some parts of parasite life cycle are still obscure, especially the liver stage which is essential for parasite development and maturation. It is likely that the parasite prevents the host hepatocyte from undergoing cell death during invasion. This is especially relevant for *Plasmodium vivax* as the hypnozoite can lay dormant in a liver cell for months even years. Identification of *P. vivax* proteins that may influence host cell death will improve our understanding of how *P. vivax* can survive for prolonged periods in the host cell during liver stage and may accelerate the development of new drugs for malaria liver stage, which is necessary for the ultimate goal of eliminating malaria.

Methods: To identify similarities based on tertiary predicted structure of proteins we used the computer algorithm i-TASSER and identified several *P. vivax* proteins which are predicted to have similar structures to human proteins involved in cell death, such as Apaf-1(Apoptotic protease activating factor 1) and cIAP-1(Cellular Inhibitor of Apoptosis). We are expressing these *P. vivax* proteins in a human liver cancer cell line, then investigating the death phenotype of cells expressing these proteins.

Results and Conclusions: These *P. vivax* proteins are successfully expressed in one human liver cell line $HepG_2$. The study of death phenotype of cells expressing these proteins is still on progress.

P-24. STRUCTURAL CHARACTERISATION OF AMPK BY CRYO-EM AND FUNCTIONAL CHARACTERISATION OF AMPK GAMMA2 AND GAMMA3 SUBUNIT ISOFORMS

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The AMPK alpha-beta-gamma heterotrimer is a highly conserved serine/threonine protein kinase that acts as a metabolic fuel sensor and is crucial for maintaining cellular energy homeostasis. It forms complexes in a 1:1:1 ratio made up of unique subunit isoform variations (alpha1, alpha2, beta1, beta2, gamma1, gamma2, gamma3) and is subject to modifications such as phosphorylation (all subunits) and NH_2-terminal myristoylation of the beta-subunit at position G2. Beta-subunit myristoylation is important for targeting AMPK to membranes during stress, acting as a "myristoyl-switch", and for some of the stimulatory effects of energy deprivation. Significant knowledge gaps exist around gamma2 and gamma3 structure, and also in structural elucidation of beta-subunit myristoylation. Additionally, gamma2 and gamma3 contain large NH_2-terminal extensions that don't exist on gamma1, and despite high throughput phosphoproteomic studies identifying >50 phosphorylation sites on the gamma2 extension, nothing is known about how this effects function.

In the present study, we have used bacterial expression constructs that co-express AMPK with *N*-myristoyltransferase to facilitate beta-subunit myristoylation. These constructs will be used in cryo-EM experiments to understand the mechanism of the beta-subunit "myristoyl-switch". Furthermore, HEK293T/17-expressed AMPK will be used in cryo-EM experiments to determine the structure of gamma2 and gamma3. We have utilised HEK293T/17-expressed AMPK to characterise AMPK allosteric activation in response to different gamma-binding activators, which corroborate with previous findings that subunit composition is an important determinant of AMPK activation. To further examine the function of the gamma2 and gamma3 NH_2-terminal extensions, mass spectrometry will be employed to identify and characterise novel phosphorylation sites.

P-25. Determination of Proliferative Activities in canine mixed mammary tumour Samples: A Comparison of Three Methods.

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Introduction: Apart from mitotic assessment and Ki67/MIB1 immunohistochemistry to identify proliferation-related antigen, other methods have been used in the past for determining proliferative cells in tissue sections i.e. DNA fluorocytometry, insitu hybridization and autoradiographic methods. The main disadvantages in these methods are that they are costly and time consuming. Nucleolar organiser regions (NORs) are known to selectively stain using silver-based histological stains. Argyrophilic nucleolar organiser regions (AgNORs) are increased in malignant tumours when compared to normal, reactive, and benign cancerous cells. In this study we have studied the diagnostic role of the mitotic figure count, Ki67 labelling index and AgNORs markers in canine mixed mammary tumours (CMMTs).

Aims: Because of similarity between human and canine mixed mammary tumours. CMMTs could provide great insight into the similar role of bio-markers in human mixed mammary tumours. Therefore, the three main proliferative bio-markers (mitotic index, Ki-67 labelling index, AgNOR count/area) were examined to determine the role of AgNORs in differentiating benign from malignant CMMTs also to determine if there is a correlation between mean AgNOR count/nuclei and mean AgNOR Area/nuclei with the mitotic figure count and with Ki67 labelling index.

Methods: Eighty cases with CMMTs were studied by histochemical, immunohistochemical, and morphometrical investigations. Mitotic index, AgNOR count/area, and Ki-67 labelling indices were examined.

Results: The present study found a significant correlation between mitotic indices and Ki-67 labelling index with different AgNORs values between benign and malignant CMMTs.

Conclusion: We conclude that, it seems to be probable to use the mean number and/ or mean area of AgNOR as an index of proliferative activity in CMMTs. The staining of AgNORs is one of the different methods which are applied for assessing the tumour tissue depending on nuclear studies, this method has many advantages to become more preferable: convenience, affordable and good Correlation with other proliferative markers. Inclusion of these markers increases the objectivity and reliability for precise diagnosis, patient management and cancer progression than the standard grade and stage alone.

P-26. TURN BACK TIME: AN INVESTIGATIVE STUDY OF HISTORICALLY DIAGNOSED LYMPHOMA CASES.

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Aim: This project involves the investigation of two historically diagnosed lymphoma cases from 1955. In order to confirm or contend the historical diagnosis, and to achieve lymphoma sub-classification, immunohistochemistry (IHC) was performed on the two cases.

Background: Historically, lymphoma cases were diagnosed by routine haematoxylin & eosin (H&E) analysis. The development of modern IHC has enabled the categorisation of the disease into specific subtypes. Lymphoma is a malignant disease, characterised by the uncontrolled proliferation of lymphoid cells; including B, T or natural killer (NK) cells. Based on the specific clinical, morphological and pathological features, lymphoma is defined as Hodgkin (HL) or Non-Hodgkin lymphoma (NHL). Although often asymptomatic, the clinical symptoms of lymphoma include superficial lymphadenopathy and hepatosplenomegaly, accompanied by systemic symptoms such as fever, night sweats, weight loss, and itching. Lymphoma is caused by the accumulation and progression of multiple genetic lesions affecting tumour suppressor genes and proto-oncogenes. Environmental factors also play an important role in the pathogenesis of lymphoma, including immunosuppression, infectious agents (EBV, HIV etc.), ultraviolet radiation and chemical exposure.

Method: The study was performed on two cases of formalin-fixed, paraffin-embedded lymph nodes. These tissue cases, provided by the RMIT University Anatomical Pathology laboratory, were both historically diagnosed with Hodgkin's lymphoma. Each of the blocks were embedded and sectioned at 4μm thickness. H&E sections of the tissues in question were assessed for features of malignancy, including Hodgkin Reed-Sternberg cells, allowing differentiation between HL and NHL. IHC stains using antibody markers were then performed to confirm or dismiss the presumptive diagnosis and to further subtype lymphoma cases.

Results: A panel of IHC markers were utilised to sub-classify the lymphoma cases in greater depth than their historical diagnosis. The IHC staining results have contended the historical diagnosis of Hodgkin's lymphoma, with both cases showing positive IHC results for Non-Hodgkin's lymphoma.

Conclusion: The use of IHC has been essential in the sub-typing of lymphomas. All cases were diagnosed as NHL subtypes.

P-27. MODELLING RESISTANCE IN MELANOMA IMMUNOTHERAPY USING CRISPR/CAS9

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Adoptive T-cell therapy (ACT) has emerged as a powerful treatment option in patients with metastatic melanoma. However, tumor cells frequently relapse from therapy by acquired resistance mechanisms such as loss of target antigen expression. Currently, it is not completely understood how the choice of target antigen influences resistance mechanisms to antigen-specific immunotherapies.

Therefore, we established CRISPR-assisted insertion of epitopes (CRISPitope), a technique that fuses a defined T-cell epitope to the C-termini of endogenous gene products. We applied CRISPitope to murine melanoma cells and tagged endogenous melanosomal TYRP1 and oncogenic $CDK4^{R24C}$ with the human $gp100_{25\text{-}33}$ epitope rendering them targetable by gp100-specific pmel-1 TCR-transgenic T cells. This enabled us to investigate melanoma escape mechanisms to ACT targeting non-essential melanosomal versus essential oncogenic antigens in direct comparison.

Using experimental mouse models, we could identify different escape mechanisms to gp100-specific immunotherapy in TYRP1 versus $CDK4^{R24C}$ melanomas. Resistance to ACT targeting TYRP1 was mainly caused by hardwired loss of antigen accompanied by a non-inflamed microenvironment or reversible downregulation of the antigen associated with an enforced melanoma phenotype switching. In contrast, $CDK4^{R24C}$ melanomas escaping ACT displayed antigen persistence and were associated with an IFN-rich inflamed tumor microenvironment. In $CDK4^{R24C}$ melanomas IFN-driven feedback inhibition by negative immune-checkpoint molecules promotes resistance to ACT despite persistent antigen expression.

Applying CRISPitope to syngeneic mouse models, we could show that target antigen choice can influence ACT resistance mechanisms, phenotype and immune contexture of melanomas in response to antigen-specific immunotherapies. Thus, our work could help to better understand acquired resistance and optimize personalized cancer immunotherapy.

P-28. DYNAMICS OF CORNEAL INFILTRATION BY IMMUNE CELLS FOLLOWING INFECTION

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Introduction: The cornea is an immune-privileged site that comprises of an epithelial and stromal compartment. Immune cell function in this space are tightly regulated to prevent damage and the loss of vision. Following infection with herpes simplex virus (HSV), T cells, a type of white blood cell, are able to infiltrate the space and control the infection.

Aims: To investigate the behaviour of T cells responding to an infection of the cornea to better understand how we can manipulate and control damage of the cornea as a result of immune responses.

Methods: Corneas of mice had the epithelium disrupted and infected with HSV KOS strain. Using microscopy and flow cytometry, we were able to track the infiltration of HSV-specific T cells into the cornea.

Results: T cells infiltrated the edges of the cornea as early as 5 days post infection and continued to mobilise towards the center, peaking at 10 days post infection. We also observed the presence of T cells in both the epithelial and stromal compartment of the cornea at memory time points, albeit at low numbers, suggesting a possibility of resident memory T cell (Trm) formation.

Conclusions: Future work with these techniques will enable us to further elucidate interactions between T cells and viral infected cells. This work will contribute to our understanding of immune interactions in the cornea in the effort of reducing blindness caused by infections.

P-29. Development of a Chromogenic In-Situ Hybridization Assay for Diagnosis of Complete and Partial Hydatidiform Moles

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Aim: To test the reliability of chromosome 17 CISH for assessing ploidy status of molar pregnancies for implementation of testing in a routine diagnostic laboratory.

Background: Molar pregnancies arise from an abnormal proliferation of trophoblastic cells that would usually develop into the placenta, and can take one of two forms; complete or partial moles. Complete moles are more common and present as haploid paternally derived tissue, while partial moles often present as triploid containing two sperm and one egg; however there is much overlap between the conditions, contributing to the difficulty in diagnosis. Classification is essential due to the risk of progression to choriocarcinoma, especially with complete moles. Currently ploidy analysis through FISH or flow cytometry reigns as the gold-standard of diagnosis, it arguably has many drawbacks specifically in relation to its inaccessibility to routine diagnostic laboratories, accentuating the need for an accurate, reliable, and accessible method of ploidy analysis that may be achieved through CISH.

Method: 50 POC samples with known ploidy status, 25 triploid and 25 diploid, were sectioned at 4 microns and stained using the automated Ventana BenchMark Ultra immunohistochemistry staining machine in conjunction with a Roche Diagnostics chromosome 17 probe and red DIG detection kit. Stains were coverslipped and assessed for ploidy. Results were compared to the external ploidy assessment undertaken by the Royal Womens Hospital Australia to measure correlation.

Results*: Results show a high correlation between the internal and external ploidy assessments, showing that chromosome 17 CISH is an adequate method for the determination of ploidy status in molar pregnancy using POC tissue. Testing was able to be seamlessly implemented into a major routine diagnostic laboratory, further highlighting the accessibility of this method compared to current methods of ploidy assessment.

*Note that results are currently based on 31/50 tests as staining is not complete, current results show 30/31 tests correlate to external ploidy assessment.

P-31. UNDERSTANDING THE ROLE OF IRF4 IN EFFECTOR TREG DIFFERENTIATION

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Regulatory T cells (Tregs) are a subset of CD4+ T cells that are essential for restraining autoreactive and inflammatory immune cells. These cells express the lineage-specifying transcription factor Foxp3, which is critical to preserving the identity and function of Tregs. In order to fully execute immunosuppression, thymus-derived Tregs must be activated through their T cell receptors (TCRs) to differentiate into functionally potent effector Tregs (eTregs), which predominantly populate non-lymphoid tissue niches and refrain various types of immune responses. The TCR-induced transcription factor IRF4 has been shown to be mandatory for the development of eTregs. Whole body and Treg-specific deletion of IRF4 results in remarkable loss of eTregs, subsequently, excessive and lethal autoimmunity. It is, however, unclear yet how exactly IRF4 facilitates eTreg differentiation.

Here using reporter mice, we tracked *Irf4* expression through Treg ontogeny. We found that *Irf4* expression is induced in thymic Foxp3^{-} Treg precursors and peaked in mature thymic Foxp3^{+} Tregs. Expression of *Irf4*, however, declined in CD62L^{hi}CD44^{lo} naïve Tregs from secondary lymphoid tissues but surged in activated Tregs in both lymphoid and non-lymphoid tissues. Analysis of IRF4-deficient mice also revealed a significant reduction of a specific thymic Treg population, suggesting a much broader role for IRF4 in Tregs besides regulating eTreg differentiation.

To investigate the IRF4-dependent eTreg-specific transcriptional program, chromatin-immunoprecipitation and sequencing (ChIP-seq) was performed on activated conventional CD4^{+} T cells (Tconvs) and Tregs to unveil the genome-wide IRF4 occupancy that is unique to Tregs. Comparison of IRF4 binding patterns reveals prominent differences, suggesting IRF4-mediated activation program is cell type-specific, possibly dependent on Foxp3 in Tregs. To address this, we performed Foxp3 ChIP-seq, which revealed a significant overlap between Foxp3- and IRF4-occupied eTreg gene loci, suggesting cooperation between these transcription factors. Given Foxp3 is stably expressed in IRF4-deficient Tregs yet unable to autonomously drive eTreg differentiation, we hypothesised that IRF4 is critical to the transcriptional activity of Foxp3. Interrogation of chromatin accessibility data, however, revealed that IRF4 may not be required for TCR-dependent chromatin accessibility but might promote Foxp3 binding through an unknown mechanism to commence the eTreg differentiation program.

P-32. DEVELOPING A MEMORY BASED DECISION MAKING TASK TO EXPLORE CORTICAL FUNCTION

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Working memory stores and manipulates information within a limited time frame. We use it without even paying attention to it such as during decision making, goal maintanence and attention. Working memory is dependent on sensory information, as well as modulation of these sensory pathways by other brain regions such as the prefrontal cortex (PFC). However, depite being a central part of our day-to-day lives, very little is known about the underlying neural activity and modulation contributing to working memory. Here, we aim to unravel the neural networks underlying working memory, explore PFC's role in a sensory-based memory task and investigate the relationship between PFC and the primary somatosensory cortex (S1). Inspired by Romo's flutter task, we are developing a behavioural task in head fixed mice, which allows us to examine the underlying neural networks of working memory in a sensory-based memory task. Once the behavioural task is succesfully implemented, we will image axonal projections from PFC to S1 with the use of two-photon calcium imaging. Furthermore, we'll make use of a combination of optogenetics and electrophsyology to examine which types of neurons in S1 receives these axonal projections from PFC. Since deficets in working memory manifests in lack of inhibitory control, impaired attention and poor memory, this study will provide invaluable insights into a range of neurological disorders, such as ADHD and Schizophrenia.

P-33. HISTONE DEACETYLASE INHIBITORS (HDACI) AS A DIFFERENTIATION THERAPY FOR NON-SMALL CELL LUNG ADENOCARCINOMA

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Impaired differentiation is a common feature of cancer. In contrast to chemotherapy, the goal of differentiation therapy is to exhaust the malignant potential of tumour cells through the reactivation of developmental programs rather than cell death.

The Switch/Sucrose Non-Fermentable (SWI/SNF) DNA remodelling complexes play a vital role in regulating cellular differentiation and have been strongly implicated in cancer. Studies suggest that SWI/SNF complexes bind to the DNA-histone scaffold and initiate transcription via the recruitment of vital cellular transcriptional machinery including histone acetyltransferases (Wilson & Roberts, 2011). Importantly, Next-generation sequencing has identified mutations in at least 10 genes encoding subunits of the SWI/SNF complexes that are recurrently mutated in cancers originating in nearly every body tissue, collectively occurring in >25% of all human malignancies (Kadoch et.al, 2013).

Lung adenocarcinoma (LAC) accounts for 40% of all lung cancers and has a 5-year survival. Mutations in SMARCA4 are believed to occur in 6% of LAC (Cancer Genome Atlas Research Network, 2014). Using a panel of SMARCA4 wildtype and mutant human LAC cell lines we show a marked sensitivity of the HDACi, Panobinostat, to SMARCA4-null cell lines. Furthermore, sustained low-dose treatment of the SMARCA4-null LAC cell line, A549, results in gene expression changes consistent with differentiation, including the down regulation of lung progenitor markers and increased expression of type II epithelial cell markers. Lastly, Panobinostat treatment significantly reduces tumour volume, extends survival and drives cell differentiation in an A549 xenograft model. Together, these data suggest that low-dose Panobinostat promotes differentiation of SMARCA4-null LAC.

P-34. EXPLORING THE INVOLVEMENT OF TRMT1 AND TRMT1L IN POST-TRANSCRIPTIONAL RNA MODIFICATIONS AND ITS LINK TO INTELLECTUAL DISABILITY.

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After the completion of the human genome project it was clear that there is no correlation between the complexity of an organism and the number of genes. A simple grape has 50 percent more genes than a human. There is however a very strong correlation between the non-coding part of the transcriptome, and the complexity of organisms. The epitranscriptome adds another layer of complexity to the transcriptome. The epitranscriptome consists out of posttranscriptional RNA modifications that play a key role in the regulation of RNA. Disruptions within the epitranscriptome are linked to numerous disorders like metabolic, cardiac, respiratory, and eurological disorders. Non-syndromic intellectual disability can be caused by mutations in TRMA Transferase 1 (TRMT1), that has been found to be responsible for the modification N2,N2-Dimethylguanosine (M22G) on position 26 of tRNAs. The aim of our research is to further investigate TRMT1 and its close paralog TRMT1L. TRMT1 was found to co-localize with mitochondria in the cytoplasm, while TRMT1L co-localizes with nucleoli in the nucleus. Additionally both change localization adter actionvation of SH-SY5Y cells with potassium chloride, which has been used as an in vitro model for Long Term Potentiation. Analysis with Liquid Column Mass Spectometry and reverse transcription mismatch signature analysis shows M22G in RNA fraction outside tRNA and changes after activation with potassium chloride. With the help of Phage Display technology we hope to develop new antibodies against RNA modifications.

P-35. DEVELOPING SMALL MOLECULE INHIBITORS OF THE AMA1/RON2 INTERACTION AS NEW ANTIMALARIAL AGENTS

Investigating the activation mechanism of the relaxin receptor RXFP1

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The peptide hormone relaxin plays key roles in cardiovascular function during pregnancy. These actions led to the use of human relaxin (H2 relaxin) as a treatment for acute heart failure. Clinical use of H2 relaxin requires constant infusion hence it would be beneficial to develop long-acting variants or small molecule agonists, especially for chronic disease treatments. However, the relaxin receptor RXFP1 has a complex mechanism of ligand-mediated activation that complicates the development of small molecules that exactly mimic the mode of relaxin-mediated activation. Relaxin binds to two sites in the large RXFP1 ectodomain but binding alone does not activate the receptor. Ligand binding stabilizes the helical conformation of a linker region between the ectodomain and the N-terminal LDLa module which reorientates the LDLa-linker to interact with the transmembrane domain (TMD) to activate the receptor. The small molecule RXFP1 agonist ML290 acts as a biased agonist by partially mimicking the action of the LDLa-linker. A better understanding of the mechanism by which the LDLa-linker and ML290 activate RXFP1 is required for structure-based design of full agonists.

In this study we have investigated the activation mechanism of relaxin and ML290 by utilizing co-expression of binding- and signaling-incompetent RXFP1 variants. We have utilized HEK cells stably expressing RXFP1-short, a variant lacking the LDLa module, which binds relaxin normally but does not signal and has full response to ML290. We have co-expressed RXFP1 TMD only constructs with or without a tethered LDLa module or a soluble LDLa-linker construct. The TMD constructs alone do not respond to relaxin but can be activated by ML290. The LDLa-linker tethered TMD when co-expressed with RXFP1-short rescues relaxin activation of cAMP while attenuating ML290 cAMP activation. This cell based activation complex can be utilized further to determine the mode of LDLa-linker mediated activation of RXFP1.

Judges and Reviewers

The Biomed Link 2018 Organising Committee blindly reviewed all abstracts submitted to Biomed Link 2018.

The Biomed Link 2018 Organising Committee would like to thank the following judges who have kindly donated their time to judge the oral and poster presentations.

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Shayarana Gooneratne

Jon Gooi

Acknowledgements

The Biomed Link 2018 Organizing Committee would like to thank the following people for their help and support throughout the organization of this conference:

Eastern Hill Academic Centre Research and Training Committee

Nora Hanafi (EHAC)

Speakers

Doctor Marguerite Evans-Galea

Professor Grant McArthur

Website Support

Eastern Hill Academic Centre

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Zouki Catering

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Thank you to all sponsors and participants who made Biomed Link 2018 possible