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BIOMEDICAL RESEARCH STUDENTS

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6TH OF NOVEMBER 2019

BRENAN HALL ST VINCENT'S HOSPITAL (MELBOURNE)

INVITED SPEAKERS

PROF. ANDREAS STRASSER

DIVISION HEAD, BLOOD CELLS & CANCER, WEHI

DR. DANIJELA MIROSA

HEAD OF STRATEGY & PATIENT SERVICES (OCEANIA CLUSTER)
TAKEDA PHARMACEUTICALS

ABSTRACT SUBMISSION OPENS: 7 JUL 2019

ABSTRACT SUBMISSION CLOSES: 7 AUG 2019

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SVI ST VINCENT'S INSTITUTE MEDICAL RESEARCH

CENTRE FOR Eye Research Australia

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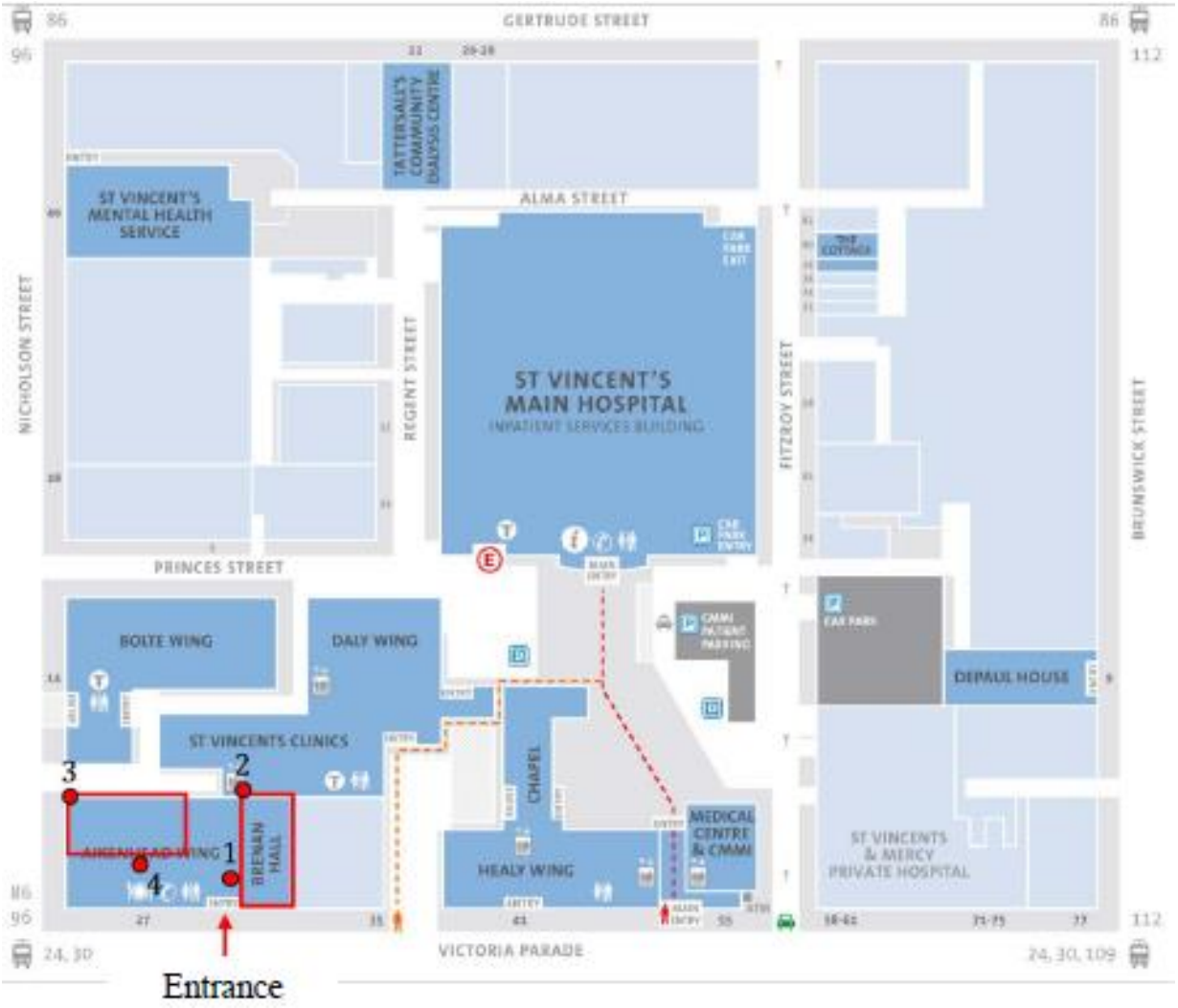
St. Vincent's Hospital Map



Points of Interests

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

Message from the Co-chairs

Dear Delegates,

Welcome to Biomed Link 2019, a student conference for biomedical students to communicate their research and network with peers from Melbourne and across Australia. The Biomed Link 2019 organising committee is made up of members from the St. Vincent's Student Society, and we are proud to provide this forum for the tenth year running, made possible by our generous sponsors.

We hope that Biomed Link 2019 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 Professor Andreas Strasser and Dr Danijela Mirosa;
- 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.

Martha Blank and Crystal Nguyen

Biomed Link 2019 Co-Chairs

Biomed Link 2019 Organising Committee

The Biomed Link 2019 conference was made possible by invaluable contributions from the committee members comprising of the following individuals:

Martha Blank

Crystal Nguyen

Co-Chairs, Biomed Link 2019

Ashley Ovens

Nicholas Waters

Jesslyn Lamtara

Daniel Urrutia Cabrera

Patrick Lam

Layal El Wazan

Alexander Murdoch

Xining Li

Luke Van Jager

Ting Ting Ge

Oliver Cucanic

Please feel free to approach any of the committee members if you have any questions or require further assistance.

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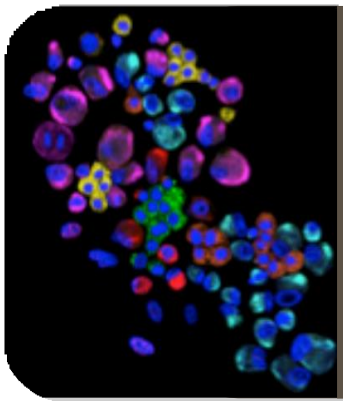

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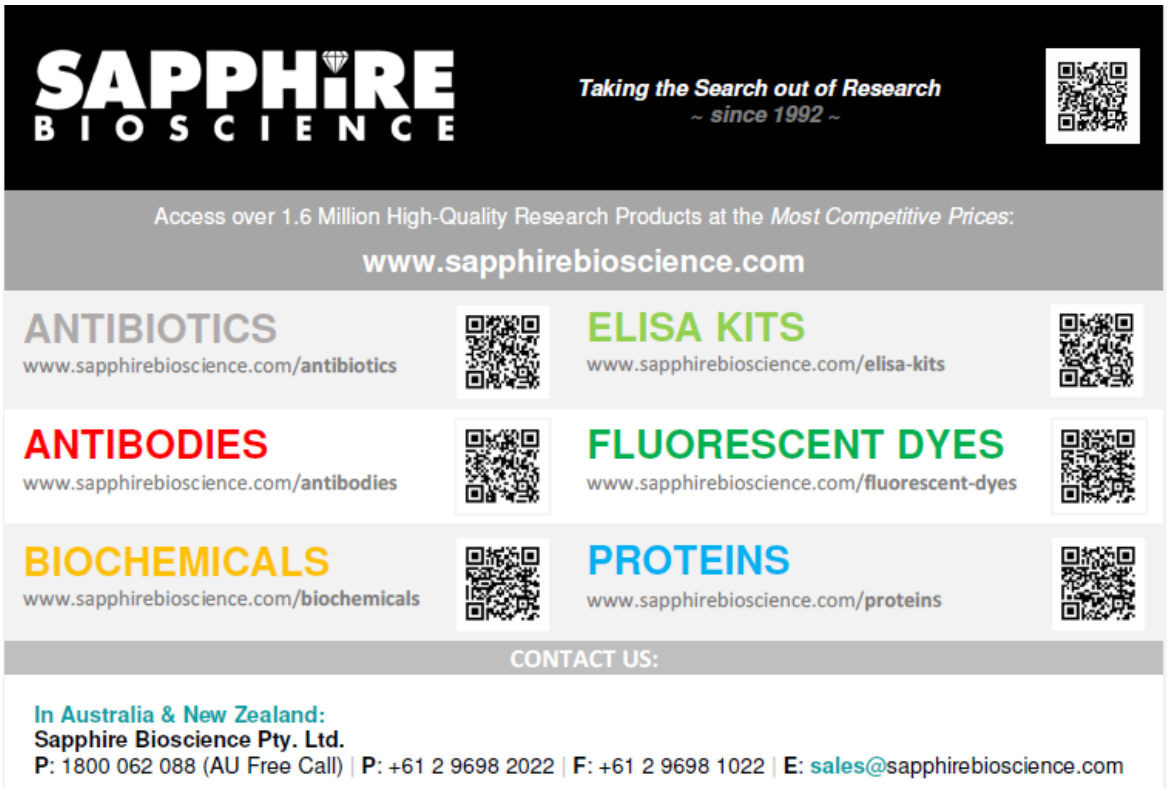

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EMBL Australia is a life science network that supports ambitious research projects and provides access to infrastructure and training to early-career Australian scientists.
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Biomed Link 2019 Session Times

8:00 – 8:30	**CONFERENCE REGISTRATION**
8:30 – 8:40	**OPENING ADDRESS** *Michael Chamberlin Lecture Theatre* **Ms Martha Blank and Ms Crystal Nguyen (Co-Chairs)**
8:40 – 8:45	**WELCOME ADDRESS** *Michael Chamberlin Lecture Theatre* **Prof. Jenny Graves** *(La Trobe University)*
8:45 – 9:30	**KEYNOTE ADDRESS** *Michael Chamberlin Lecture Theatre* **Prof. Andreas Strasser** *(Walter and Eliza Hall Institute of Medical Research)* ***How does the tumor suppressor p53 protect us from cancer and how can it be targeted therapeutically?***
Session 1 **9:30 – 10:30**	**3 MINUTE-THESIS** *Michael Chamberlin Lecture Theatre* Judges: Dr. Amanda Edgley (University of Melbourne) and Ms Nicola Peniguel (St. Vincent's Institute of Medical Research)
10:30 – 11:30	**MORNING TEA & POSTER PRESENTATIONS** *SESSION 1 Brennan Hall*
Session 2 **11:30 – 13:00**	**ORAL PRESENTATIONS** *SESSION 1 Michael Chamberlin Lecture Theatre* Judges: Prof. David Vaux (Walter and Eliza Hall Institute of Medical Research), Prof. Natalie Sims (St. Vincent's Institute of Medical Research) and Dr. Andrew Sutherland (St. Vincent's Institute of Medical Research) **Lokman Pang** *Investigating the Role of GP130/STAT3 Signalling in Intestinal Barrier Function* **Catriona Nguyen-Robertson** *CD1A-Restricted T cells: A Unique Population of Lipid Reactive Cells* **Winnie Tan** *Monoubiquitination of FANCI:FANCD2 Locks Itself to DNA to Form Filaments* **Wei Shern Lee** *Genetic and Transcriptional Analysis of Brain Malformation at Single-Cell Resolution* **Meaghan Griffiths** *High Dose Radiotherapy Directly Damages the Uterus and Compromises Fertility* **Fabrizzio Horta** *Female Age Affects the DNA Repair Capacity of Oocytes in IVF Using a Controlled Model of Sperm DNA Damage* **Ana Rita Leitoguinho** *The Role of Ventx Homeobox Gene During Human Haematopoietic Development*
13:00 – 14:30	**LUNCH & POSTER PRESENTATIONS** *SESSION 2 Brennan Hall*

Session 3 **14:30 – 16:00**	**ORAL PRESENTATIONS** *SESSION 2 Michael Chamberlin Lecture Theatre* Judges: Prof. Jenny Graves (LaTrobe University), Dr. Jörg Heierhorst (St. Vincent's Institute of Medical Research) and Dr. Catherine Drinkwater (BioCurate) **John Nguyen** *The Cellular Microenvironment Supports Muscle Stem Cell Proliferation and Regeneration* **Marija Dinevska** *Targeting Ion Channels with FDA-Approved Therapeutics to Regulate Invadopia Formation/Activity in Glioma Cells* **Christina Gangemi** *Light-Induced Proliferation of Pancreatic Beta Cells Mediated by OPN3* **Andrew Treller** *Investigating Vascular Dysfunction in Friedreich's Ataxia Using Human Induced Pluripotent Stem Cells* **Marija Simic** *Anti-LRP6 Antibody Prevents Myeloma Induced Bone Disease and Increases Bone Strength* **Mariah Alorro** *Selective Stat3 Inhibition in the Tumour Microenvironment Restricts Gastrointestinal Tumour Growth* **Hoang Kim Ngan Le** *Characterising the Role of Il-36G in the Development of Gastric Cancer* **Ryan O'Keefe** *Targeting Tuft cells and Innate Lymphoid Cells in Gastric Cancer*
16:00 – 16:30	**AFTERNOON TEA & POSTER PRESENTATIONS** *SESSION 3 Brennan Hall*
16:30 – 17:10	**KEYNOTE ADDRESS** *Michael Chamberlin Lecture Theatre* **Dr. Danijela Mirosa** *(Takeda, Head of Strategy & Patient Services, Oceania Cluster)* ***Life after the lab - from bench to business and beyond***
17:10 – 17:30	**PRESENTATION OF AWARDS** *Michael Chamberlin Lecture Theatre*
17:30 – 19:00	**CLOSING RECEPTION** *Brennan Hall*

Welcome Address

Prof. Jenny Graves – *Vice-Chancellor's Fellow & Distinguished Professor, Ecology, Environment & Evolution, La Trobe University*

8:40 – 8:45

Michael Chamberlin Lecture Theatre

Jenny Graves made seminal contributions to the understanding of mammalian genome organization and evolution, exploiting the genetic diversity of Australia's unique animals as a source of genetic variation to study highly conserved genetic structures and processes. Her studies of the chromosomes and genes of kangaroos and platypus, devils (Tasmanian) and dragons (lizards) has shed light on the organisation, function and evolution of mammalian genomes, and led to influential new theories of the origin and evolution of human sex chromosomes and sex determining genes. She is (in)famous for her prediction that the human Y chromosome is disappearing. She made critical discoveries that the epigenetic silencing of mammalian X chromosomes occurred by transcriptional inhibition, and is mediated by DNA methylation. Her recent work, in collaboration with scientists at the University of Canberra, explores epigenetics and sex determination, using reptile models that have sex chromosomes, but undergo sex reversal at high temperatures.

Jenny has published more than 430 scientific works, including 4 books. She was elected a Fellow of the Australian Academy of Science in 1999 and served on the Academy Executive, first as Foreign Secretary, then as Secretary for Education. She is 2006 L'Oreal-UNESCO Laureate, and has received many awards for her work, including the MacFarlane Burnet Medal for research in biology, and an AO

Keynote Address

Professor Andreas Strasser – *Division Head: Blood Cells and Blood Cancer, Walter and Eliza Hall Institute*

"How does the tumour suppressor p53 protect us from cancer and how can it be targeted therapeutically?"

8:45-9:30

Michael Chamberlin Lecture Theatre

Professor Andreas Strasser is a cancer researcher at the Walter and Eliza Hall Institute trained in cell biology, immunology and molecular oncology. He has made major contributions to the discoveries that defects in cell death can cause cancer, autoimmune disease and impair the response of cancers to chemotherapy. His current work aims to reach a detailed understanding of the molecular control of programmed cell death. His team is exploiting this knowledge to develop novel treatments for cancer and autoimmune diseases that directly activate the cell death machinery.

Dr Danijela Mirosa - *Head of Strategy & Patient Services for Oceania Cluster, Takeda Pharmaceuticals*

"Life after the lab - from Bench to Business and Beyond"

16:30-17:10

Michael Chamberlin Lecture Theatre

Dr Danijela Mirosa is the Head of Strategy & Patient Services for Oceania Cluster, Takeda Pharmaceuticals. She has over 15 years of "bench to business" biopharmaceutical experience across Rare and Specialty disease areas. Following the completion of her PhD, Dani spent more than 6 years as a translational research scientist in the Australian biotechnology sector, specializing in experimental recombinant antibody drugs against oncology and inflammatory disease targets. Having gained this experience in early stage drug development, she then expanded her career into the commercial side of the industry. She has held numerous marketing roles across diverse therapeutic areas such as Immunology, Neurology, Haemophilia, Oncology and Ophthalmology, and more recently she was Head of Strategy & Operations, Australia/New Zealand for Shire prior to its acquisition by Takeda in early 2019. Her qualifications include a PhD in Biomedicine and an MBA from Melbourne Business School.

Biomed Link 2019 3MT Session

3 MINUTE-THESIS *Michael Chamberlin Lecture Theatre*
9:30-10:30
Judges: Dr. Amanda Edgley (University of Melbourne) and Ms Nicola Peniguel (St. Vincent's Institute of Medical Research)

Jonathan Kim (P-7)
Developing a Symptom Monitoring Tool for Patients with Upper Gastrointestinal Cancer

Ayeshah A. Rosdah (P-8)
Novel DRP1 Inhibitors for Cardioprotection

Annie Tan (P-18)
Developing a Core Outcome Set to Measure the Effects of Pain Management During Labour and Childbirth

Divyangana Rakesh (P-24)
Coupling of Resting State Networks in Adolescent Problematic Substance Use and Depression

Sina Mansour L. (P-26)
Structural Connectome Fingerprinting

Sarah Sandford (P-35)
Intestinal Immune and Stromal Cell Responses to Acute Chronic Viral Infection

Annalisa Carli (P-48)
DCLK1: A Novel Promoter of Gastric Cancer Progression

Samantha Davis (P-52)
Non-Specific IgA Inhibits HIV Broadly Neutralizing Antibody (bnAB) Functions

Motahhareh Tourchi R (P-54)
Investigating the Role of Timeless in Breast Cancer

Thomas Chow (P-59)
Assessing the Growth of Human-Derived Osteosarcoma Tumour Cells Within Hydrogel Scaffolds for the Development of a 3D-Printable Osteosarcoma Organoid

Celeste Piotto (P-62)
Enhancing Tissue Regeneration by Promoting Regulatory T Cell Accumulation into Damaged Tissue

Sarah Thomas Brome (P-63)
Targeting Neuroinflammation in Parkinson's Disease

Lap Hing Chi (P-65)
BMP4 Inhibits Breast Cancer Metastasis Independent of Tumour SMAD4

Monica Hu (P-70)
Looking Towards Gene Therapy: Characterisation of Ex Vivo Aav2.Cyp4V2 Gene Therapy in Human Retinal Explants

Françios Olivier (P-71)
No More Mr. Fun Guy: In Vitro Modelling of Candida Albicans-Induced Death in Human Macrophages

Diannita Kwang (P-72)
Retinoic Acid Receptor γ Activity in Endothelial Cells Regulates Haematopoiesis and Bone Marrow Niche Integrity

Clea Grace (P-73)
Hoxa1-Overexpression in Serial Murine Bone Marrow Transplants Induces Thrombocytopenia and Haematopoietic Stem Cell Myeloid-Priming

Oral Presentation Abstracts

Session 2
11:30-13:00
Pages 18-24

Session 3
14:30-16:00
Pages 25-32

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

O-1 INVESTIGATING THE ROLE OF GP130/STAT3 SIGNALLING IN INTESTINAL BARRIER FUNCTION

Lokman Pang[1,2], Jennifer Huynh[1,2], Matthias Ernst[1,2], Ashwini Chand[1,2]

[1]Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, Australia
[2]La Trobe University, School of Cancer Medicine, Bundoora, VIC, Australia

Introduction
Colorectal cancer (CRC) is the third most common fatal malignancy worldwide, with 40-50% patients dying from this disease. As its prevalence continues to rise, advances in treatments are urgently needed to alleviate the morbidity and mortality associated with CRC. The intestinal epithelium provides a physical and biochemical barrier against commensal and pathogenic microorganisms. Perturbations in barrier function promote chronic inflammation, which can drive tumorigenesis and alter responsiveness to anti-cancer therapies.

Aims
The Signal Transducer and Activator of Transcription 3 **(STAT3)** is a key driver in the progression of inflammation-associated CRC. However, the role for STAT3 in intestinal barrier function is yet to be delineated. Here we study the role of STAT3 in intestinal barrier function during chemically-induced colitis.

Method
We utilised two different mouse models to partially ablate STAT3 protein or its genetic expression *in vivo*. One mouse model harbours a truncated GP130 receptor **(GP130ΔSTAT/+)** to reduce GP130-dependent STAT1/3-mediated activation. The second mouse model carries a Doxycycline-inducible Stat3 short-hairpin RNA **(shSTAT3)** for the reversible genetic silencing of *Stat3*. These mice were then challenged with the chemical irritant Dextran Sulfate Sodium (DSS) and the intestinal barrier function was assessed using an *in vivo* FITC-dextran permeability assay. Colonic tissues were harvested and analysed via qRT-PCR and Western blotting.

Results
We demonstrate that partial STAT3 deletion significantly increases the susceptibility to DSS-induced colitis, indicated by i) decreased intestinal barrier function, ii) severe weight loss and iii) histological damage. We further identify that reduced barrier function is accompanied by altered expression of the *Reg3b and Reg3g* antimicrobial genes, as well as reduced expression of the tight junction Claudin proteins.

Conclusion
Together, our data suggest STAT3 activity is essential for the maintenance of intestinal barrier function. Therapeutically targeting the GP130/STAT3 signalling cascade in intestinal epithelial cells, and selectively manipulating barrier function, may pose as a potential strategy to alter responsiveness to chemo- and immuno-therapy in CRC patients.

O-2 CD1A-RESTRICTED T CELLS: A UNIQUE POPULATION OF LIPID REACTIVE T CELLS

Catriona V Nguyen-Robertson[1,2], Scott JJ Reddiex[2], Willem Van Der Byl[3], Janice MH Cheng[1,4], Spencer J Williams[4], Fabio Luciani[3], Dale I Godfrey[1], Daniel G Pellicci[2]

[1]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC, Australia
[2]Cellular Immunology Group, Murdoch Children's Research Institute, Parkville, VIC, Australia
[3]Kirby Institute, University of New South Wales, Kensington, UNSW, Australia
[4]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC, Australia

Introduction
In contrast to conventional T cells that recognise peptide antigens presented by MHC molecules, a group of innate-like T cells recognise lipid antigens presented by MHC-like CD1 family members: CD1a, CD1b, CD1c and CD1d. Recent studies have suggested that CD1a-restricted T cells comprise a unique T cell population in human blood and may also play a unique functional role in skin. They represent up to 10% of peripheral blood T cells, yet relatively little is known about the role of these cells in the immune system.

Aims
We aimed to isolate human CD1a-restricted T cells to investigate their phenotype and function *ex vivo*. We also aimed to characterise the interaction between their T cell receptors and CD1a presenting both foreign- and self-lipid antigens.

Methods
We produced CD1a tetramers to label and isolate CD1a-restricted T cells from human blood and skin samples using flow cytometry.

Results
Interestingly, we have shown that CD1a-restricted T cells recognise non-self-lipid antigens, particularly dideoxymycobactin (DDM), a lipid antigen derived from *Mycobacterium tuberculosis*, can also be autoreactive to self-lipids. These T cells that cross-react between multiple lipid antigens (both self and non-self) are unlike conventional T cells that recognise one, unique antigen. Not only did we observe reactivity of these T cells to foreign lipids from bacteria, they also reacted to oils from skincare products and endogenous human lipids, suggesting that they contribute not only to defence in infection, but also allergies to skincare products and autoimmunity.

Additionally, we have defined the T cell receptor (TCR) usage of both self- and foreign-lipid-reactive CD1a-restricted T cells, demonstrating that while they exhibit a diverse TCR repertoire, there is some biased usage of certain variable genes. Phenotypic analyses and RNA-sequencing of these cells revealed that they are distinct from other unconventional, innate-like T cells, such as natural killer T cells, thereby distinguishing CD1a-restricted T cells as a unique population of unconventional T cells. For example, they do not express innate-like markers such as CD161 and IL-18R, nor do they have a semi-invariant TCR.

Conclusions
While many studies have grouped lipid-reactive T cells, this study shows that CD1a-restricted T cells represent a distinct population of immune cells. They therefore may play a unique role in infection, allergy, and autoimmunity by reacting to various lipid antigens. This represents an important step forward in characterising CD1a-restricted T cells, and further understanding their role in infection and autoimmune responses.

O-3 MONOUBIQUITINATION STABILIZES FANCI:FANCD2 FILAMENT ON DNA TO INITIATE DNA REPAIR

Winnie Tan[1,2], Sylvie van Twest[1], Vincent Murphy[1], Andrew Leis[3], Michael Parker[4], Andrew Deans[1,2]

[1]Genome Stability Unit, St. Vincent's Institute of Medical Research, Fitzroy, Victoria, 3065 Australia
[2]Department of Medicine (St. Vincent's Health), The University of Melbourne, Australia
[3] Bio21 Institute, Parkville, Victoria, 3052 Australia
[4]Structural Biology Laboratory, Bio21 Institute, Parkville, Victoria, 3052 Australia

Introduction
Fanconi anaemia (FA) is a rare, genetic disease characterized by defective DNA repair that results in chromosomal instability, congenital defects, bone marrow failure and cancer pre-disposition. The FA core complex assembles in response to DNA damage and monoubiquitinates FANCI and FANCD2 to signal downstream DNA repair.

Aims
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. Using a novel Avi-tag ubiquitin, we report the first purification of monoubiquitinated, DNA bound FANCI:FANCD2 complex.

Results
We show that various double-stranded DNA-containing structures could robustly stimulate mono-ubiquitination of FANCI:FANCD2, but that single-stranded DNA does not. 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, but the non-ubiquitinatable FANCD2-K521R mutant was not. Western blotting of the EMSA gels confirmed that the gel shifted band contains both FANCI and FANCD2 proteins. To verify that monoubiquitination stabilizes FANCI:FANCD2 on DNA, we purified the first bi-mono-ubiquitinated FANCI:FANCD2 complex bound to DNA. Unexpectedly, we show that purified monoubiquitinated, DNA bound FANCI:FANCD2 complex assembles into a filamentous oligomer along the length of dsDNA, using negative-stain electron microscopy. Here, we report an initial structure of the monoubiquitinated FANCI:FANCD2 complex bound to double-stranded (ds) DNA determined by single-particle electron microscopy. Monoubiquitination of both FANCI and FANCD2 are required to "lock" the heterodimer on dsDNA. Finally, we show that the reversal of the monoubiquitination by the USP1:UAF1 deubiquitinase only occurs when DNA is disengaged. These results suggest a previously unrecognized role of FANCI:FANCD2-DNA filament in control of ubiquitination and deubiquitination processes, thereby regulating DNA repair signaling.

Conclusion
We show that mono-ubiquitination of FANCI:FANCD2 complex "locks" itself to the DNA, recapitulates how this complex regulates DNA damage in cells. Deubiquitination of FANCI:FANCD2 is inhibited when DNA is present, suggesting the role of another nuclease specific to ubiquitinated form of FANCI:FANCD2 complex to excise the DNA prior to deubiquitination. 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.

O-4 GENETIC AND TRANSCRIPTOMIC ANALYSIS OF BRAIN MALFORMATIONS AT SINGLE-CELL RESOLUTION

Wei Shern Lee[1,2], Sarah EM Stephenson[1,2], Saskia Freytag[3], A Simon Harvey[4], Wirginia Maixner[4], Melanie Bahlo[5], Richard J Leventer[4], Paul J Lockhart[1,2]

[1]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC
[2]Department of Paediatrics, University of Melbourne, Parkville, VIC
[3]Harry Perkins Institute for Medical Research, Perth, WA
[4]Department of Neurology, Royal Children's Hospital, Parkville, VIC
[5]Walter and Eliza Hall Institute, Parkville, VIC

Introduction
The human brain is genetically mosaic. Pathogenic somatic mutations occurring during brain development can cause brain malformations in children, with symptoms including cerebral palsy, developmental delay and drug-resistant focal epilepsy. Microscopically, brain malformations are characterised by focal disruption of cortical layering and the presence of dysmorphic neurons. Affected children may require surgical removal of the affected cortex for seizure control. To date, the genetics of brain malformations and the biology of dysmorphic neurons remain incompletely understood.

Aim
To understand the genetics and transcriptomics of brain malformations at single-cell resolution using patient-derived brain tissue.

Methods
For genetic analysis, gDNA was extracted from paired resected brain tissues (n=30) and peripheral blood (n=30) for deep sequencing (>500x depth) to identify somatic mutations. Immunohistochemistry was performed to visualise dysmorphic neurons on brain sections followed by laser capture microdissection to isolate dysmorphic neurons. For transcriptomic analysis, dysplastic (n=8) and normal brain tissues (n=4) were analysed using single-nucleus RNA-sequencing (snRNA-seq).

Results
We identified low allele frequency (1.5%~6.3%), brain-specific somatic mutations in 10 of 30 cases. Further investigation in one case showed a 'mutation gradient' across the affected cortex, with varying levels of somatic mutation load in different regions. This mutation gradient was positively correlated to the density of dysmorphic neurons, and the highest mutation load is found in regions with the strongest epileptic discharge. We used laser capture microdissection to isolate dysmorphic neurons and showed that somatic mutation can only be found in dysmorphic neurons but not in normal neurons. We next performed snRNA-seq to capture the transcriptomics of dysplastic and normal brain specimens at single-cell resolution. We captured 47,706 nuclei, generating a dataset of 25 cell clusters representing different brain cell types. We identified a unique cluster enriched in the dysplastic brain specimens, which may represent the dysmorphic neurons. This cluster exhibits high expression of transcripts not shared by other clusters, highlighting the potential of using these transcripts to delineate the biology of dysmorphic neurons.

Conclusions
Low allele frequency somatic mutations in brain malformations are limited to dysmorphic neurons, which exhibit different density across the affected cortex. High density of dysmorphic neurons is indicative of strong epileptic discharge. Transcriptomic analysis using snRNA-seq revealed cell cluster potentially representing the dysmorphic neurons and provided genes of interest for further investigation. Our results offer novel insights into the genetics and transcriptomics of brain malformations, which will be applicable to the broader research in epilepsy and brain development.

O-5 HIGH DOSE RADIOTHERAPY DIRECTLY DAMAGES THE UTERUS AND COMPROMISES FERTILITY

Meaghan Griffiths[1], Amy Winship[1], Karla Hutt[1]

[1] Ovarian Biology Laboratory, Department of Anatomy and Developmental Biology, Stem Cell and Development Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria

Introduction

As cancer survival rates consistently improve, addressing the long-term off-target effects of cancer treatments is becoming increasingly important. Approximately 80% of cancer survivors will experience diminished fertility. Despite advances in fertility preservation techniques including oocyte or ovarian tissue cryopreservation, pregnancy success rates are still lower in cancer survivors compared to the general population, or other women seeking assisted reproduction technologies. The uterus is the major organ in the female reproductive system that is responsible for providing the nourishing environment for foetal growth. In order to establish pregnancy, the endometrium (the innermost lining of the uterus) must be receptive, and only becomes receptive in a short window with each menstrual cycle in humans. If the uterine environment during pregnancy is abnormal then pregnancy complications can arise, including preterm birth, low birth weight infants or small for gestational age offspring.

Aims

We aimed to develop an animal model to define the impacts of radiotherapy on the uterus and its ability to establish and maintain pregnancy in the presence of healthy embryos.

Methods

Adolescent (4-6 week old) female mice exposed to high dose irradiation (7Gy), or non-irradiated control (n=4-8/group) were ovariectomised before hormonal stimulation to induce endometrial receptivity, or embryo transfer surgery using healthy unexposed donor mouse embryos.

Results

Within hours of irradiation, markers of DNA damage (yH2AX), cell death (CC3 and TUNEL) and the intrinsic apoptosis pathway (Puma) localise to the irradiated adolescent mouse uteri, with Puma mRNA significantly elevated 24 hours post-irradiation (n=4/group, $p<0.05$). Additionally, in response to hormonally induced endometrial receptivity, irradiated mice have smaller uteri ($p<0.05$), decreased expression of estrogen receptor alpha ($p<0.05$) and a trend for decreased progesterone receptor expression ($p<0.1$). Irradiated mice that receive healthy donor embryos have a similar number of early implantation sites to non-irradiated controls, however uteri are white in appearance, suggesting impaired vascularisation in response to pregnancy establishment. To investigate this further we are assessing later stage embryos and employing ultrasound imaging to determine whether uterine and umbilical artery blood flow are impaired following radiotherapy.

Conclusions

We have shown for the first time that the intrinsic apoptosis pathway is activated in the mouse uterus post-irradiation. Furthermore, mice exposed to high dose radiotherapy have reduced hormone receptor expression during receptivity, yet have similar numbers of implantation sites compared to non-irradiated controls. Further investigations will define whether the mouse uterus exposed to radiotherapy can sustain healthy pregnancy throughout gestation and give rise to healthy offspring.

O-6 FEMALE AGE AFFECTS THE DNA REPAIR CAPACITY OF OOCYTES IN IVF USING A CONTROLLED MODEL OF SPERM DNA DAMAGE

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Introduction
The reproductive lifespan in women declines with age predominantly due to poor oocyte quality. This leads to decreased reproductive outcomes for older women undergoing assisted reproductive technology (ART) treatments compared to young women. Aging and oocyte quality have been clearly associated with aneuploidy, but factors determining oocyte quality remain unclear. DNA repair activity prior to embryonic genomic activation is considered of maternal origin, with maternal transcripts and proteins controlling DNA integrity. With increasing maternal/oocyte age, stored mRNAs decrease, which could result in diminished efficiency of DNA repair and/or negative effects on embryo development, especially considering the presence of DNA damage.

Aims
The aim of this study was to examine the effects of female age on the DNA repair capacity of mouse oocytes using controlled radiation model to induce sperm DNA damage.

Methods
Oocytes from two groups of mice (Young: 5-8 weeks, n=15; Old: 42-45 weeks, n=15) were inseminated with control non-irradiated spermatozoa and spermatozoa exposed to 1Gy and 30 Gy of radiation and assessed for fertilisation and blastocyst formation using time-lapse imaging technology (ESCO, MIRI). DNA damage response (DDR) was assessed in zygotes and 2 cell embryos produced in control and 1Gy groups using γH2AX immunofluorescence labelling and confocal microscopy. Replicates (n=5) using GV and MII oocytes from each age group, were analysed for expression of 91 DNA repair genes (Fluidigm Biomark HD system).

Results
Fertilization rates were similar among study groups ($p>0.05$). Blastocyst formation rates from young and old females fertilised with control spermatozoa were significantly higher than those fertilised using irradiated sperm cell. [(Young; Control: 86.95%, 1GY: 33.33%, 30GY: 0.0%) (Old; Control: 70.37%, 1GY: 0.0%, 30GY: 0.0%); *$p<0.001$*]. Between age groups, 1GY samples showed a significant decrease in the blastocyst rate in old females compared to young females (Young; 1GY: 33.33%, Old; 1GY: 0.0, *$p=0.0166$*). There was decreased DDR in embryos from old females (*$p<0.05$*) compared to young, regardless of spermatozoa group. Down-regulation in expression of 21 and 23 genes was found in older GV and MII oocytes respectively (*$p<0.05$*).

Conclusion
This study demonstrated a significant age-related decrease in DNA repair capacity of mouse oocytes. Fertilisation can occur with sperm populations with medium and high DNA damage but subsequent embryo growth is affected, and to a greater extent with aging females, supporting the theory that oocyte DNA repair capacity decreases with age. The assessment of oocyte DNA repair capacity could be a useful clinical diagnostic tool.

O-7 THE ROLE OF *VENTX* HOMEOBOX GENE DURING HUMAN HAEMATOPOIETIC DEVELOPMENT

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Introduction
The *Ventx* genes are non-clustered homeobox transcription factors that confer a ventral phenotype on mesodermal cells in the developing embryo. *Ventx* genes are conserved in vertebrates but have been lost in rodents. In the human haematopoietic system, *VENTX* promotes myeloid differentiation and is expressed in some acute myeloid leukaemias (AML).

Aims
Use human pluripotent stem cells to model blood development upon conditional overexpression of *VENTX* at different times.

Methods
Here we used Doxycycline (DOX) inducible human pluripotent stem cell line to overexpress *VENTX* during haematopoietic differentiation. We performed RNA-seq analysis and gene expression analysis in combination with ATAC and ChIP-sequencing to look for genomic targets of *VENTX* transcription factor.

Results
We found that *VENTX* overexpression in hPSC impaired mesoderm formation, but *VENTX* enforced expression after mesoderm commitment resulted in the emergence of an increased percentage of immature blood cells that co-expressed CD90 and CD34. These cells displayed high clonogenic capacity in methylcellulose, but only after DOX was removed from the media. This suggested that *VENTX* expression held cells in a non-proliferative state. Transcriptional profiling revealed increased expression of *HOXA* genes in haematopoietic cells following *VENTX* induction, consistent with their immature phenotype. Conversely, genes involved in myeloid differentiation were down regulated during *VENTX* overexpression, as were genes involved in proliferation, such as *MYC* and *MYB*. ATAC-sequencing demonstrated that *VENTX* closes selected chromatin loci, in particular areas targeted by HOXB13 and CDX transcription factors.

Conclusions
We hypothesise that *VENTX* might act as a transcriptional suppressor during haematopoietic differentiation and we are currently investigating the genomic targets of *VENTX*, combining ATAC-seq and ChIP-seq with the transcriptional profiling. In summary, *VENTX* overexpression generated immature blood cells in a quiescent state, preventing their proliferation and differentiation into myeloid lineages.

O-8 THE CELLULAR MICROENVIRONMENT SUPPORTS MUSCLE STEM CELL PROLIFERATION AND REGENERATION

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Introduction

Skeletal muscle has a remarkable potential to regenerate following injury, a process mediated by the tissue resident population of muscle stem cells (MuSCs). Following injury, MuSCs must effectively double their cellular content to proliferate, requiring large amounts of new biomass in the form of DNA/RNA, proteins and phospholipid membranes. Previous work has identified a process of 'metabolic reprogramming' in MuSCs as they switch from a quiescent to a proliferative state, shifting from a reliance on oxidative phosphorylation to glycolysis. In addition to generating ATP, glycolysis has been shown to support cellular proliferation by supplying precursors required for the synthesis of new biomass suggesting a critical role for the local metabolic microenvironment.

Aims

The aims of this study were to first characterise the metabolic microenvironment of injured and regenerating skeletal muscle and then to identify key metabolites which regulate the processes of MuSC proliferation and differentiation.

Methods

The right tibialis anterior (TA) muscle of male C57Bl/6 mice (n=12) was injured via intramuscular injection of the myotoxin $Bacl_2$ (50 µl of 1.2% solution). Uninjured (day 0) and injured muscles (day 3 and day 7) were extracted and used for metabolomic LC-MS/MS analysis. Following annotation and normalisation, several hundred metabolites were identified as differentially expressed. From this list, two metabolites were chosen for further *in vitro* analyses. The immortalised C2C12 myoblast cell line was used to assess the effect of the metabolites of interest on proliferation and differentiation, using a combination of raw cell counts, immunofluorescence and Western immunoblotting.

Results

A metabolic signature of MuSC-mediated regeneration was obtained for uninjured, 3- and 7-days post injury, with over 500 polar metabolites identified. The most highly enriched pathway at day 3 was the 'Pentose Phosphate Pathway' (nucleotide synthesis). Culture in the presence of hypoxanthine (a metabolite involved in nucleotide synthesis) significantly increased the rate of proliferation reducing the mean doubling time from 20.3hrs to 17.0hrs, 95% CI [27.43-31.57] and [23.92 – 25.05] respectively. The increase in proliferation came at the expense of differentiation (p=0.0011) with cells cultured in hypoxanthine exhibiting a significant reduction in myotube area, width and number.

Conclusions

Here we have generated the first ever metabolomic signature of regenerating skeletal muscle. We have identified hypoxanthine, a critical metabolite in the regulation of MuSC proliferation and have shown its supplementation can increase the proliferative capacity of cultured cells. These results have important implications for both cell culture and skeletal muscle regeneration.

O-9 TARGETING ION CHANNELS WITH FDA-APPROVED THERAPEUTICS TO REGULATE INVADOPODIA FORMATION/ACTIVITY IN GLIOMA CELLS

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Introduction
Glioblastoma (GBM) is the most malignant and prevalent type of primary brain cancer representing 45-50% of all primary central nervous system tumours. Despite the current standard therapy, glioblastoma patients face a poor prognosis with a median survival of 14.6 months. Dynamic actin-rich protrusions known as *invadopodia* have been proposed to mediate the highly invasive nature of glioma cells, through the coordinated action of signalling proteins and proteases that lead to the proteolytic degradation of the extracellular matrix. Importantly, ion channels have also been linked to a pro-invasive phenotype and may be involved in the invadopodia activity of cancer cells.

Aims
In this study, we aimed to examine a panel of 20 FDA-approved drugs with known ion channel targets for their cytotoxic efficacy and anti-invadopodia activity in glioblastoma cell lines.

Method
To form the foundation of our study, we analysed Oncomine mRNA and DNA genomic data, to determine if ion channels are overexpressed in glioblastoma. This was extended by correlating invadopodia regulator and ion channel gene expression with survival in glioma patients, using the SurvExpress biomarker and survival database tool. Cell viability assays were used to screen and shortlist the panel of 20 FDA-approved drugs. Zymographic analysis and invadopodia matrix degradation assays were conducted to examine the potential anti-invasive effect of the shortlisted drugs alone and in combination with radiation and temozolomide.

Results
Three FDA-approved drugs were selected based on cytotoxicity for further analysis: flunarizine dihydrochloride (FL), econazole nitrate (EN) and quinine hydrochloride dihydrate (Q). These drugs demonstrated a reduction in invadopodia-mediated FITC-gelatin degradation, with a decreasing trend in metalloproteinase-2 (MMP-2) secretion. Importantly, treatment with the three drugs led to a significant reduction in radiation/temozolomide-induced invadopodia activity.

Conclusion
The dualistic 'cytotoxic' efficacy and 'anti-invasive' activity exhibited by EN and to a lesser extent FL and Q, suggests that targeting ion channels is worthy of further research for inhibiting GBM malignancy, with a potential for future clinical translation in combination with radiotherapy and temozolomide treatment.

O-10 LIGHT-INDUCED PROLIFERATION OF PANCREATIC BETA CELLS MEDIATED BY OPN3

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Introduction
The loss of insulin-producing beta cells is the primary etiology of diabetes mellitus. Given their low rate of basal proliferation, methods to induce beta cell regeneration remain a desirable treatment for diabetes. Light has yet to be explored as an approach to promote regeneration of this cell population, nevertheless it has the advantage of providing specific and temporal stimulation. In the mammalian eye, light not only mediates vision and entrains the circadian clock, but it was also found to activate major survival and proliferative signalling pathways. Gene expression data from our lab and others showed that photoreceptor proteins are not only expressed in the retina but also in pancreatic tissue.

Aims
(i) To explore the effect of visible light on beta cell survival and proliferation, and (ii) characterise the photoreceptor-related genes and signalling pathways required for this effect.

Methods
Primary murine pancreatic islets were isolated from animals and human islets sourced from donors. EdU incorporation assays were performed to detect proliferating cells. Beta cell-specific proliferation was further analysed using insulin as a marker. Downstream signalling pathway activation was characterised by immunoblot analysis. Beta cell identity and function was validated through Glucose Stimulated Insulin Secretion (GSIS) assays. Specific light requirements and photoreceptor-related gene expression was determined using RT-PCR and a subsequent loss-of-function mouse model.

Results
In primary murine and human islets, blue-green light illumination was found to be sufficient for the activation of proliferation. In human islets, light induced an increase in Insulin+ beta cells, whereas other islet cell types were unaffected. Optical stimulation did not negatively affect the GSIS capacity of beta cells, suggesting retained cell identity and function. Most notably, there was enhanced insulin secretion under high glucose conditions after illumination in primary human islets. Immunoblot analysis detected increased phosphorylated Akt (pAkt). This was abolished in the presence of light and the Akt inhibitor GSK-69069350, indicating that islet illumination promotes pro-survival signalling. RT-PCR revealed the expression of light-sensing proteins, most notably OPN3, a mammalian receptor of the opsin family. In an $OPN3^{-}/^{-}$ mouse model, a light-induced increase in proliferation and pAkt levels was not observed, confirming the role of OPN3 as the primary photoreceptor mediating this light-induced proliferative response.

Conclusions
Our results reveal a novel endogenous light-sensing mechanism in pancreatic islets. This sensitivity points to a diverse role of opsins in non-light exposed tissues, and suggests new optical strategies for the regeneration of beta cells in disorders such as diabetes.

O-11 INVESTIGATING VASCULAR DYSFUNCTION IN FRIEDREICH'S ATAXIA USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Introduction
Friedreich's ataxia (FRDA) is an autosomal recessive disease due to an unstable expanded GAA repeat mutation of the frataxin gene, resulting in reduced levels of mitochondrial protein frataxin. The leading cause of mortality in FRDA is cardiomyopathy.Currently, there are no treatments which can cure, or even slow FRDA disease progression. FRDA autopsy reports have suggested abnormalities of the small coronary arteries which can potentially be the cause of cardiomyopathy in FRDA patients. However, the mechanisms underlying the pathogenesis of the diseased blood vessels in FRDA remain unknown.

Aims
(1) To generate endothelial cells from induced pluripotent stem cells derived from FRDA patients (FRDA-iPSCs) and CRISPR-corrected isogenic controls. (2) To assess the endothelial and mitochondrial functions in endothelial cells derived from FRDA- and isogenic control-iPSCs.

Method
Cardiac tissue biopsies from FRDA patients were stained with haematoxylin and eosin, as well as immunostained for vascular cell markers. FRDA- and isogenic control-iPSCs were differentiated into endothelial cells and CD31 positive endothelial cells were purified by Fluorescence Activated Cell Sorting. Frataxin gene and protein expression levels were determined by RT-qPCR and Dipstick assay, respectively. Proliferative and angiogenic potential of endothelial cells were assessed by Ki-67 staining and tubulogenesis assay, respectively. Cell viability (Annexin V and propidium iodide staining), mitochondrial membrane potential (JC-1 staining), and mitochondrial superoxide level (MitoSOX Red staining) of endothelial cells under basal condition and in response to oxidative stress (hydrogen peroxide treatment) were assessed by flow cytometry.

Results
Cardiac tissue of FRDA patients displayed focal interstitial fibrosis and increases in endothelial cell proliferation in some small blood vessels, creating a non-uniform intimal layer that partially occluded the vessels. Compared to FRDA-iPSCs, CRISPR-corrected isogenic control iPSCs displayed an ~3-fold increase in *FXN* mRNA and ~6-fold increase in frataxin protein confirming the reversal of the disease phenotype. Endothelial cells derived from FRDA- and isogenic control-iPSCs expressed the endothelial cell markers (CD31, VE-cadherin and von Willebrand factor) and formed capillary-like tube structures when seeded on Matrigel. Compared to isogenic controls, endothelial cells derived from FRDA-iPSCs have a higher mitochondrial membrane potential ($n=4$, $P<0.05$) and equivalent level of mitochondrial superoxide under basal and oxidative stress conditions. Analyses on cell viability and proliferation are ongoing.

Conclusion
Endothelial cells derived from FRDA-iPSCs exhibit abnormal mitochondrial function. This *in vitro* pre-clinical vascular model may provide a valuable platform for future drug discovery to develop novel treatment for FRDA-induced cardiomyopathy.

O-12 ANTI-LRP6 ANTIBODY PREVENTS MYELOMA INDUCED BONE DISEASE AND INCREASES BONE STRENGTH
★ ***BioCurate travel award***

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Introduction
Multiple myeloma (MM) is a plasma cell cancer that leads to bone destruction through both increased bone resorption and decreased bone formation. This bone loss leads to high fracture rates and increased mortality. Anti-resorptive treatments prevent further bone loss, but patients continue to experience fractures. Therefore, new therapies which promote bone formation are required to rebuild skeletal structure and prevent fractures in patients with myeloma. LRP6 is a Wnt ligand co-receptor which drives Wnt stimulated differentiation of osteoblasts. Anti-LRP6 antibodies promote Wnt signalling and have been shown to increase bone mass, however they are yet to be explored in the setting of myeloma.

Aims
To determine whether the Wnt promoting agent, anti-LRP6, prevents myeloma-induced bone loss and reduces fractures.

Methods
Mice injected with eGFP expressing 5TGM1 murine myeloma cells and their naïve counterparts, were treated twice weekly with anti-LRP6 or the control isotype (100mg/kg i.v.) for 21 days. MicroCT, vertebral compression testing, histomorphometry and FACS were utilised to determine bone structural changes, bone strength, cellular mechanisms, and tumour burden in response to anti-LRP6 treatment respectively.

Results
Naive mice treated with anti-LRP6 had a 23% increase in vertebral bone volume per tissue volume (BV/TV) compared to their controls ($p<0.05$). 5TGM1-burdened mice demonstrated a 23% reduction in vertebral BV/TV compared to naïve controls ($p<0.05$). This loss in bone volume was associated with a 34% reduction in the maximum load required to failure ($p<0.0001$). Anti-LRP6 treatment prevented the structural bone loss seen in 5TGM1-burdened mice, through increasing vertebral BV/TV by 33% ($p<0.01$). This in turn increased the maximum load to failure by 32% ($p<0.01$), almost reaching equivalent strength to naïve controls in this model. Similar improvements in bone volume were also shown in the femur, however anti-LRP6 antibody did not alter bone formation or tumour growth.

Conclusions
Although anti-LRP6 treatment did not reduce tumour burden, it did prevent the development of myeloma induced bone disease, improved bone structure and strength. This study defines a therapeutic strategy superior to current approaches, which will reduce fractures and improve quality of life in patients with myeloma.

O-13 SELECTIVE STAT3 INHIBITION IN THE TUMOUR MICROENVIRONMENT RESTRICTS GASTROINTESTINAL TUMOUR GROWTH

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Introduction
The signal transducer and activator of transcription 3 (STAT3) is key a transcription factor often found to be overexpressed in tumours, and is associated with the development of cancer hallmarks such as sustained proliferation and avoidance of cell death. In fact, the pro-tumorigenic role of intrinsic Stat3 signalling within tumour cells is well characterised across a number of different cancers, including gastrointestinal cancers. However, the influence of Stat3 among the non-tumour cells that infiltrate the tumour microenvironment is less explored.

Aims
This project aims to elucidate the role(s) of STAT3 in the non-tumoural compartment of the tumour microenvironment.

Methods
We developed a shStat3 transgenic mouse that allows for the conditional knock-down of STAT3. The shStat3 was crossed with the $gp130^{F/F}$ mutant gastric cancer mouse. The shStat3 mice were also injected subcutaneously with MC38 murine colon cancer cells. Variations of this model were used for bone marrow chimera experiments, and testing against the Stat3 small molecule inhibitor BBI-608. Tissues from these experiments were subjected to protein and RNA analysis, by western blot and q-RT-PCR respectively. The immune-profiles of the excised tumours were also interrogated by FACS analysis.

Results
Systemic Stat3 reduction in the $gp130^{F/F}$ mice decreased tumour burden. Importantly, Stat3 knockdown in the non-tumoural compartment alone significantly inhibited allografted-MC38 tumour growth. Bone marrow chimera experiments confirmed the hematopoietic compartment as the main drivers of this anti-tumour effect. Furthermore, an increase of monocytic ($Ly6C^{+}Ly6G^{-}$) cells was observed in Stat3-knockdown tumour allografts, an affect recapitulated with pharmacological Stat3 inhibition. Functionally, the Stat3-knockdown monocytes exhibited reduced immunosuppressive gene signature expression.

Conclusion
Our data provides compelling evidence of the therapeutic value of specific Stat3 targeting as a novel therapy option against gastrointestinal cancers. Strikingly, the anti-tumoural responses were shown to be at least partially mediated through immune cells such as the ($Ly6C^{+}Ly6G^{-}$) monocytes, highlighting a key role for the immune environment in the responsiveness to anti-Stat3 therapies. The latter findings warrant further investigation and could lead to major implications on the stratification of patients that might respond to such therapies.

O-14 CHARACTERISING THE ROLE OF IL-36G IN THE DEVELOPMENT OF GASTRIC CANCER

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Introduction
Gastric cancer (GC) is one of the leading causes of cancer-related deaths worldwide, whereby *Helicobacter pylori* is a major predisposition factor which leads to chronic inflammation. There is a lack of effective treatments since high recurrence of GC are has been reported, thus increasing a demand to develop new therapeutic avenues to treat this disease. During progression of GC, inflammation is tightly regulated by immune cells and factors they produce called cytokines. Our preliminary data indicates that IL-36G cytokine expression is elevated in gastric tumours from patients and high IL-36G levels correlate with poorer patient survival rate. We postulate that IL-36G could be a pro-tumour cytokine which can be targeted as a novel treatment.

Aims
The aim of this project is to characterise the effects IL-36G elicits on gastric cancer cell proliferation, and whether these pro-tumour effects can be inhibited by its natural antagonist, IL-36RN.

Methods
The expression levels of members of the IL-36G family of cytokines were measured by qPCR analysis in three gastric cancer cell lines (GCCs) (AGS, MKN45, MKN1). The effects of IL-36G on GCC proliferation was assessed by colony forming assays. IL-36G signalling can be inhibited by the natural antagonist IL-36RN. Therefore, GCCs were stimulated with IL-36G in the presence of IL-36RN to ascertain if the antagonist can inhibit IL-36G-mediated GCC proliferation. The effects of IL-36G stimulation on ERK phosphorylation in the absence and presence of IL-36RN was also determined by Western Blot.

Results
The mRNA expression levels of IL-36A, IL-36B, IL-36G and its heterodimeric receptors (IL-36RN, IL-1RAP) were detected in AGS, MKN45 and MKN1 cell lines. IL-36G mounted a proliferative response at a concentration of 0.1 ng/ml, in comparison to untreated cells. The natural antagonist IL-36RN stimulation was found to inhibit IL-36G-induced colony formation at a concentration of 30 ng/ml. IL-36G was also found to induce ERK2 phosphorylation in GCCs.

Conclusions
Here, we report that IL-36G induces cell proliferation and ERK phosphorylation which can be suppressed by its natural antagonist, IL-36RN. Overall, results from this study provide a solid premise to evaluate IL-36G-based therapeutics for the treatment of GC using *in vivo* animal studies.

O-15 TARGETING TUFT CELLS AND INNATE LYMPHOID CELLS IN GASTRIC CANCER

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Introduction
Gastric cancer is the third leading cause of cancer-related deaths, and accounts for 900,000 deaths annually. Tuft cells are a rare subset of mucosal epithelial cells that are significantly increased during gastric tumorigenesis, and serve as a major source of IL25 within the tumour microenvironment. The production of IL25 promotes IL13 secretion by activated type 2 Innate Lymphoid Cells (ILC2s), and results in a feed-forward loop that promotes tuft cell development through the IL25/IL13 signal transduction pathway.

Aims
Here we assess the therapeutic potential of targeting tuft cells and ILC2s during gastric cancer.

Methods
To better understand the role of tuft cells and ILC2s in gastric tumour progression, we utilized the $Gp130^{F/F}$ mouse model of spontaneous intestinal-type gastric cancer to assess tuft cell and ILC2 numbers. To study the therapeutic benefit of targeting tuft cells and ILC2s interactions, we genetically ablated tuft cells in treated tumour-bearing $Gp130^{F/F}$ mice or treated them with a neutralising anti-IL25 antibody.

Results
We observed a significant increase in tuft cells and ILC2s in the blood and gastric tumours of $Gp130^{F/F}$ mice compared to wild-type (WT) controls. These results were consistent with increased Il13 and Il25 gene expression in $Gp130^{F/F}$ tumours compared to WT tissue. Accordingly, tuft cell ablation significantly impaired tumour growth and ILC2s in $Gp130^{F/F}$ mice, and reduced Il13 and Il25 gene expression within tumours.
Likewise, anti-IL25 treatment in $Gp130^{F/F}$ mice led to significantly smaller tumours and reduced tuft cell numbers in these mice. In vitro analysis of gastric tumour organoids similarly demonstrated that treatment with anti-IL25 suppressed tumour organoid growth, while stimulation with IL13 enhanced organoid growth.

Conclusion
Together, our results suggest tuft cells and ILC2s form a positive feed-forward loop that drives gastric tumour development through an IL25/IL13 signalling cascade. Inhibition of this pathway therefore provides a promising therapeutic approach for the treatment of gastric cancer.

Poster Presentation Abstracts

Judging Times

Session 1
10:30-11:30

Session 2
13:00-14:30

Session 3
16:00-16:30

Poster presentations will be held at the Brennan Hall

P-1 REGULATION OF FETAL TESTIS DEVELOPMENT BY FGF9 AND THE MAPK SIGNALLING PATHWAY

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Introduction

In mammals, male or female development depends on the formation of a testis or ovary, a process involving a series of transcriptional and signalling events in the gonads of developing embryos. Testis development begins with the expression of sex-determining region Y (*Sry*) and its primary target gene, Sry-box 9 (*Sox9*), driving the commitment of supporting cells to form Sertoli cells. Fibroblast growth factor 9 (FGF9) is involved in this process through the formation of a positive feedback loop with SOX9. Signalling from the Sertoli cells, including via FGF9, then directs germ cells to commit to the male fate, undergo mitotic arrest and male germline differentiation. A number of signalling pathways involved have been identified, including the Mitogen Activated Protein Kinase (MAPK) pathway.

Aims

As FGF's are known to signal through the MAPK pathway in other tissues, this project aimed to determine if FGF9 signals through the MAPK pathway in the developing testis by exploring how inhibition of FGF9 or MEK1/2 signalling may affect the development of Sertoli and germ cells.

Methods

Embryonic day (E)12.5 mouse testes from Oct4GFP transgenic mice were cultured with increasing doses of FGF9 or MEK1/2 inhibitors for 72 hours. Oct4GFP specifically marks germ cells allowing germ cell survival and gross testis morphology to be monitored in culture and for isolation of germ cells. The impacts of FGF9 or MEK1/2 signalling inhibition on Sertoli cell proliferation, testis cord structure, germ cell mitotic arrest and expression of genes important in testis and male germline development were determined using whole gonad imaging, flow cytometry, immunofluorescent imaging and qRT-PCR.

Results

At the gross morphological level, testis cord structure was affected by both treatments, with germ cells appearing to escape from the cords. Inhibition of MEK1/2 signalling inhibited germ cell mitotic arrest, marked by a significant increase in germ cell proliferation, which was accompanied by a significant reduction in Sertoli cell proliferation. By comparison, FGF9 inhibition resulted in a significant reduction in Sertoli cell proliferation but did not appear to affect the ability of germ cells to enter mitotic arrest.

Conclusions

This data indicates a role for MEK1/2 signalling in testis development, including entry of germ cells into mitotic arrest and ensuring the integrity of testis cord structure. While FGF9 signalling was required for the development of the testis cords, it was not essential for entry of germ cells into mitotic arrest after E12.5, indicating that other signalling may be involved.

P-2 TRANSFORMING GROWTH FACTOR-BETA (TGFB) INCREASES AIRWAY FIBROSIS AND REACTIVITY TO METHACHOLINE

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Introduction and Aim
Transforming growth factor-beta (TGFβ) is a pleiotropic cytokine involved in a multitude of processes including wound healing and fibrosis. It is central to chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). Broncho-constriction results in the release of TGFβ, but the potential autocrine effects of this increased local TGFβ on airway contraction remains unknown. This study hypothesised that TGFβ leads to increased airway reactivity to methacholine (MCh).

Methods
We utilised a transgenic mouse model that over-expresses active TGFβ specifically in the lungs following doxycycline (Dox) administration. Dox was added to the drinking water for 8 weeks prior to experimentation, with normal water given to control mice. Lung plethysmography was used to measure increases in airway resistance to MCh *in vivo* before serum and BAL were collected for TGFβ ELISA. Formalin-fixed paraffin-embedded sections were also prepared, dewaxed and scanned using the Histoindex for analysis of collagen using Fibroindex. Separate control and Dox mice were used to prepare Precision Cut Lung Slice (PCLS) for *in vitro* methacholine challenge, visualising airway contraction as changes in the area.

Results
Dox treatment to induce TGFβ over-expression increased TGFβ levels in BAL, but not serum. This was associated with ~25% higher lung collagen deposition, predominantly localised around the airways. TGFβ over-expression increased *in vitro* contraction of intrapulmonary airways to MCh (maximum %reduction in airway area: control 41±5%; Dox 75±9%; n=5/group, $p<0.05$).

Conclusion
Elevated TGFβ levels in the lung result in greater airway contraction to MCh, despite increased airway fibrosis. This model can now be used to provide insights into the mechanisms underlying the potential contribution of TGFβ to airway hyperresponsiveness and may identify novel therapeutic targets to oppose excessive airway contraction in chronic lung diseases.

P-3 RELAXIN INHIBITS REMODELLING OF COLLAGEN GELS BY HUMAN LUNG FIBROBLASTS

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Introduction
Lung fibrosis is a progressive and irreversible feature of many chronic lung diseases, such as idiopathic pulmonary fibrosis and silicosis, and is associated with increased levels of transforming growth factor β1 (TGF-β1). Contraction of collagen gels seeded with fibroblasts (collagen "remodelling") is a well-established *in vitro* measure of fibrosis, used to assess the potential efficacy of anti-fibrotic agents. The effects of relaxin (RLX), a hormone of pregnancy with known anti-fibrotic properties, have yet to be tested in this assay.

Aim
To assess the anti-remodelling effects of RLX on cell- and TGF-β1-mediated collagen gel contraction using 3D collagen gels seeded with primary human lung fibroblasts.

Methods
Collagen gels were prepared using rat-tail tendon collagen and human primary lung fibroblasts obtained from unused donor lungs (Alfred Hospital). Cells were seeded in gels at a range of cell densities ($0.25x10^6$ – $1x10^6$ cells/mL). Reductions in gel area over 72h in the absence (control) and presence of TGF-β1 (4pM, 40pM and 400pM) and/or RLX (10nM, 100nM and 1μM) were measured using Image J and normalised to initial gel area.

Results
Preliminary data suggest that contraction of collagen gels is both cell- and time-dependent. The presence of fibroblasts alone at densities $>0.25x10^6$ cells/mL caused reductions in control gel area of up to 72% by 72h. In gels seeded at $0.5x10^6$ cells/mL, fibroblast-mediated reductions in area were further increased by 8% in gels treated with TGF-β1 (40 pM). In the presence of RLX (1μM), the contraction of both untreated control gels and TGF-β1- treated gels was attenuated by 18% and 29% respectively.

Conclusions
RLX inhibits cell- and TGF-β1-mediated contraction of collagen gels containing human lung fibroblasts. The inhibition of collagen remodelling by RLX supports future experiments using fibroblasts obtained in disease context, such as from explanted lungs from patients undergoing transplant for IPF. Validation of efficacy in opposing gel contraction as well as other relevant measures of fibrosis such as collagen synthesis would support clinical translation of RLX as a novel treatment for fibrotic lung disorders.

P-4 THE ROLE OF THE PRORENIN RECEPTOR IN MAINTAINING THE PRIMORDIAL FOLLICLE POOL

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Introduction

Women are born with a finite pool of follicles. Several of these are activated every month which leads to the ovulation of one oocyte and atresia of the remaining follicles. Premature ovarian insufficiency (POI) occurs when a woman under 40 years of age begins menopause due to a short supply of ovarian follicles. Unfortunately, the cause for POI is predominantly idiopathic with underlying mechanisms being unknown.

Autophagy is a process used to remove unwanted components from a cell. When autophagy is commenced a double membrane vesicle will be formed called an autophagosome. This will then engulf damaged cellular materials before fusing with a lysosome. When it associates with the autophagosome membrane, light-chain (LC) 3BI will be modified via addition of a lipid group into LC3BII. For this reason, LC3B is commonly used as an indicator of autophagic activity. Interestingly, autophagy is important for follicle atresia as it can induce apoptosis within somatic cells.

The prorenin receptor (PRR) is a component of the renin angiotensin system. While it can be activated by prorenin or renin, it can also act alone. Autophagy is one pathway which the PRR can induce independently. Previous studies in the kidneys have shown increased levels of autophagosomes and accumulation of LC3B when the PRR has been knocked out in mice. However, the link between the PRR and follicle atresia within the ovary remains unknown.

Aims

To determine if a knockout of the PRR will lead to a reduced follicle pool due to increased autophagy.

Methods

A Cre-lox system has been used to knockout the PRR in the somatic cells of the mouse ovary. An oocyte count was performed to determine if there was a reduction in the follicle pool which could lead to POI. Transmission electron microscopy (TEM) was done to identify autophagic vesicles and their placement within the ovary. Ovaries were also subject to immunofluorescent staining to determine the presence of LC3B.

Results

The oocyte count found a significant loss of activated oocytes in the knockout compared to the control. TEM appeared to show an increase in autophagosomes in somatic cells within knockout ovaries. However, a cell count of LC3B positive cells using immunofluorescence showed that there was no significant difference in autophagy between the two conditions.

Conclusions

We have successfully demonstrated that a loss of the PRR leads to a decreased follicle pool. However, as the tests for autophagy showed conflicting results, more work is needed to determine whether autophagy in ovarian somatic cells is changed due to PRR loss.

P-5 DIRECT CARDIAC REPROGRAMMING USING CRISPR-ACTIVATION TECHNOLOGY

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Introduction
Cardiovascular disease is the leading cause of death worldwide. Ischaemic heart disease is characterized by the formation of non-contractile fibrotic scar tissue as a consequence of the death of heart cells (cardiomyocytes), which have limited regenerative ability. Direct cardiac reprogramming is a promising strategy that is capable of replenishing the lost cardiomyocytes after injury by directly converting cardiac fibroblasts into new cardiomyocytes. Previous studies have demonstrated the ability to directly reprogram human fibroblasts into induced cardiomyocytes both *in vitro* and *in vivo.* However, current direct cardiac reprogramming methods rely on viral-based systems to deliver cardiac-specific transcription factors and have limited clinical applicability due to risks of random insertion mutagenesis and immunogenicity. The CRISPR-activation system, consisting of single guide RNAs (sgRNAs) and a 'dead' Cas9 protein (dSpCas9), presents an exciting opportunity for site-specific overexpression of endogenous cardiogenic transcription factors for direct cardiac reprogramming and offers a promising alternative to viral delivery of cDNA for efficient direct cardiac reprogramming.

Aims
(1) To optimize and select individual sgRNAs, capable of robust overexpression of *GATA4, MEF2C, TBX5* and *HAND2 (GMTH)* in HEK293 cells and human fibroblasts.
(2) To determine if human fibroblasts can be directly reprogrammed into induced cardiomyocytes by overexpressing *GMTH* simultaneously using the CRISPR-activator system, dSpCas9-VPR.

Methods
Two different sgRNAs were designed for each of *GATA4, MEF2C, TBX5* and *HAND2* and commercially synthesized as expression cassettes. HEK293 cells or human fibroblasts were co-transfected with the dSpCas9-VPR plasmid and individual sgRNAs targeting the specific gene. Gene overexpression was quantified using RT-qPCR two days post-transfection, with data dictating the sgRNAs used in multiplex transfections for direct reprogramming in aim 2. Selected sgRNAs simultaneously targeting *GMTH* were then co-transfected with the dSpCas9-VPR plasmid, with early cardiac-specific gene expression quantified at 2 and 14 days post-transfection. Protein expression of cardiac markers was analysed using flow cytometry and immunocytochemistry at 14 days post-transfection.

Results
Designed sgRNAs induced modest gene overexpression of *GATA4, MEF2C, TBX5* and *HAND2* when compared to dSpCas9-VPR control, in HEK293T cells. Gene and protein analyses in human fibroblasts are currently ongoing.

Conclusions
Co-transfection of individual sgRNAs with dSpCas9-VPR can induce modest overexpression of targeted genes in HEK293T cells.

P-6 MODELLING THE MITOCHONDRIAL DISEASE SENGERS SYNDROME USING HUMAN EMBRYONIC STEM CELLS

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Introduction
Mitochondria play an important role in cellular processes including energy generation, cellular signalling, 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 AGK proteins being encoded. In hESCs with mutations, the TIM22 complex was not detected by immunoblotting. Furthermore, mutant hESCs presented with disrupted ATP synthesis. 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.

P-7 DEVELOPING A SYMPTOM MONITORING TOOL FOR PATIENTS WITH UPPER GASTROINTESTINAL CANCER

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Introduction
Upper gastrointestinal cancers (UGIC) are characterised by high symptom burden, poor survivorship due to a lack of improving treatments and an overall poor health-related quality of life (HRQOL). As the five-year relative survival rate for different types of UGIC (pancreatic, oesophageal and stomach) are 9.8%, 22% and 30.3% respectively, the focus to improve HRQOL should be redirected to monitoring symptoms of patients with UGIC.

Aim
The aim of this study is to develop a symptom monitoring tool based on the perceptions of relevant healthcare professionals.

Methods
Six focus groups (three clinicians-based and three allied health and nursing-based) were conducted to identify actionable symptoms to include in the symptom monitoring tool, gradient clinical alert thresholds and implementation challenges. These symptoms were extracted from validated Patient Reported Outcome Measures (PROMs) on UGIC. A deductive framework analysis was then conducted across the six transcripts from the focus groups.

Results
The results were that symptoms were deemed actionable to a degree but not necessarily important to include in the symptom monitoring tool such as burping and taste change, and that a gradient clinical threshold to alert the relevant healthcare professional(s) via a cancer care coordinator (CCC) was required to be individualised for each symptom on the appropriate response to seek emergency department or medical advice. Furthermore, on developing the symptom monitoring tool, the 'categories' cultivated from the framework analysis include 'prompting the focus group', 'reducing patient burden on completing the tool', 'implementation challenges' and 'modularising the symptoms'.

Conclusions
Future directions would be to incorporate the 'categories' to generate a draft symptom monitoring tool for patients with UGIC to complete in a think-aloud interview setting. By further refining, the symptom monitoring tool based on the perceptions of healthcare professionals and patients with UGIC, the tool will be rolled out to patients with UGIC across 11 hospital sites as part of a larger registry-based randomised controlled trial known as PROpatient. In conclusion, by establishing the categories qualitatively derived from the focus groups with the relevant healthcare professionals, can patients with UGIC assuredly complete the symptom monitoring tool, and receive the healthcare they require to improve their HRQOL and potentially their survivorship.

P-8 NOVEL DRP1 INHIBITORS FOR CARDIOPROTECTION

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Introduction
Heart disease is the leading cause of death and disability worldwide. Therapeutic strategies for protecting the heart from ischaemia-reperfusion injury are needed. Myocardial IRI is characterised by excessive mitochondrial fission accompanied by mitochondrial dysfunction, ultimately leading to cardiomyocyte death. Genetic inhibition of the mitochondrial fission protein Drp1 has been shown to prevent cell death induced by acute ischaemia-reperfusion injury. To date, Mdivi-1 is the only known small molecule described to inhibit Drp1. However, Mdivi-1 is poorly water-soluble and has a high IC_{50} (1-10 µM). Moreover, a recent study also reported that Mdivi-1 poorly inhibits the GTPase activity of human Drp1 and failed to induce mitochondrial elongation in mammalian cell lines. This study suggested that Mdivi-1 might not be a specific inhibitor of Drp1. Collectively, the specificity and bioavailability issues have been the main obstacles in the clinical development of this cardioprotective intervention targeting Drp1.

Aim
To identify novel Drp1 inhibitors which are potent, selective and directly bind to human Drp1.

Methods
The high-throughput computational screening was conducted to identify lead compounds that can interact with the druggable pockets of Drp1. Surface plasmon resonance was conducted to assess direct binding of lead compounds with human Drp1 protein, followed by a colourimetric GTPase activity assay to confirm their inhibitory action on Drp1 enzymatic activity. Mitochondrial fission was evaluated by analysing mitochondrial morphology of wild-type and Drp1 knock-out murine embryonic fibroblasts (MEFs) treated with lead compounds at 5, 10 and 50 µM for 24 hours. Cytoprotection effect of lead compounds will be examined *in vitro* and *in vivo* models of myocardial ischaemia-reperfusion injury.

Results
Through a computational screen of 4.5 million compounds coupled with surface plasmon resonance and GTPase assay, we have identified three lead compounds, namely DRP1i1, DRP1i2 and DRP1i3, that bind directly to human Drp1 and significantly inhibit the GTPase activity of human Drp1 ($p<0.05$). On the other hand, Mdivi-1 does not bind to human Drp1. Study on mitochondrial morphology is ongoing.

Conclusion
DRP1i1, DRP1i2 and DRP1i3 are drug-like small molecules that exhibit specific inhibition towards human Drp1 and could have cardioprotective effect with better potency and specificity, and potentially fewer side effects than Mdivi-1.

P-9 VESTIBULAR DYSFUNCTION FOLLOWING COCHLEAR IMPLANTATION

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Introduction

Dizziness is a common perioperative complication after cochlear implant surgery. Currently, its aetiology is poorly understood. Previous studies suggest that dizziness symptoms may be related to otolith changes after surgery, as evidenced by reductions in saccular function during the late post-operative period. However, it is difficult to assess saccular function in the short term due to a lack of available bedside tests. A possible solution may be to study utricular function instead to assess otolith function in the perioperative period, using non-invasive tests such as the Subjective Visual Vertical (SVV).

Aims

To describe the incidence of perioperative dizziness after cochlear implantation and the changes that occur in otolith function pre- and post-surgery. Then, to correlate otolith dysfunction with the perception of dizziness.

Methods

We designed a prospective cohort study to look for evidence of otolith dysfunction after cochlear implant surgery. Patients were seen pre-operatively, 1 day, 1 week, and 6 weeks after surgery.

At each time point, subjective and objective clinical measures were collected. Firstly, patients were interviewed using pre- and post-operative questionnaires to assess pre-existing balance problems and new onset dizziness after surgery. Then, patients were asked to wear a Virtual Reality headset containing a validated version of the SVV test, Curator SVV, which can be used by the bedside to measure utricular function.

Results

Based on preliminary results, the incidence of perioperative dizziness varied across all three time intervals; at day 1 post op, 10/31 patients (32.3%) experienced balance problems, compared to 13/29 (44.8%) at 1 week, and 3/18 (16.7%) at 6 weeks. Follow up SVV data was then compared to pre-operative baseline values and categorised as 'changed' or 'unchanged'. Of the 10 patients who experienced dizziness at day 1 post op, 8/10 (80%) of the patients had significant changes to the SVV mean, versus 9/13 (69.2%) at 1 week and 3/3 (100%) at 6 weeks. Overall, a strong correlation was found between subjective dizziness and SVV changes at 1 week after surgery, but not at 1 day or 6 weeks post op.

Conclusions

Roughly a third of patients can experience transient dizziness symptoms shortly after cochlear implant surgery, with most cases resolving by 6 weeks. These symptoms are strongly correlated with SVV changes at 1 week after surgery, suggesting that otolith dysfunction may be a possible cause behind patients' balance problems.

P-10 DFP TREATMENT AMELIORATES ASPECTS OF THE DYSTROPHIC PATHOLOGY IN *MDX* MICE

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Introduction and Aim

Duchenne muscular dystrophy (DMD) is a genetic disorder characterised by progressive muscle wasting and weakness leading to premature death. Our preliminary findings identified a loss in iron homeostasis in dystrophic skeletal muscles and a relationship between accumulation of iron and severity of the dystrophic pathology. Thus, we aimed to alleviate aspects of the dystrophic pathology in mdx mice with Deferiprone (DFP), an iron chelator to attenuate muscle atrophy.

Methods

Deferiprone (DFP) was administered in a short-term study (4 weeks) to investigate the effect of DFP on skeletal muscle metabolism. 3 week old male mdx mice received either normal drinking water (CONTROL, n=10) or drinking water containing DFP (150mg/kg/d; n=10). Following whole body functional assessments, hindlimb muscles along with other organs were excised, weighed and structural and biochemical analysis were performed.

Results

DFP-treated mdx mice displayed a reduction in reactive oxygen species in the diaphragm (DHE -25%, $P < 0.05$) and collagen infiltration (−26%, $P < 0.05$). DFP treatment did not reduce ferritin levels but resulted in a significant decrease in a muscle specific iron containing protein, myoglobin.

Conclusions

DFP supplementation attenuated the progression of the dystrophic pathology. Although DFP did not alter functional performance, the reduction in fibrosis is clinically relevant for increasing the efficacy of therapies for DMD.

P-11 INVESTIGATING THE TUMOUR SUPPRESSOR FUNCTIONS OF TP53

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Introduction

The tumour suppressor, TP53, functions to prevent cancer development. Upon detection of cellular stress, TP53 induces the transcription of a large number of genes that function across multiple cellular processes including apoptosis, cell cycle arrest, and cellular senescence. The factors that determine which one of these processes will dominate in a particular cell type, and ultimately dictate the fate of that cell, is unclear. Understanding these cellular decisions has important implications for cancer therapy, since understanding how a particular cancer cell will respond to TP53 activating therapies, such as DNA damaging chemotherapies, could inform the choice of therapy.

Aims

We aim to characterise the response of a range of human tumour derived cell lines of different cell origins containing wild-type p53 to p53 activation and to determine the cellular factors which govern the nature of their response.

Methods

To examine which factors control the fate decision of a particular cell, we have characterised nine human cancer derived cell lines with wild-type TP53 for their response to nutlin-3a (an MDM2 inhibitor, the critical negative regulator of TP53). Using flow cytometry, we assessed apoptotic cell death, cell cycle stages and cellular senescence, as well as protein levels of TP53 and a key transcriptional target of TP53, namely P21.

Results

Upon TP53 activation, we found that each cell line undergoes one main response in a dose dependant matter. Interestingly though, a small proportion of cells within a cell line can respond differently.

Conclusions

This research provides a foundation to further investigate and determine what causes cells to commit to different responses following TP53 activation.

P-12 ELUCIDATING THE ZINC-BINDING MECHANISM OF STREPTOCOCCUS PNEUMONIAE ADCAII

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Introduction
Streptococcus pneumoniae is a globally significant human pathogen responsible for more than a million deaths annually. To colonise and persist within the host, the bacterium must acquire the transition metal ion zinc [Zn(II)], which is poorly abundant in the host environment. In *S. pneumoniae*, Zn(II) import is facilitated by the ATP-binding cassette transporter, AdcCB, and two Zn(II)-specific solute binding proteins (SBPs), AdcA and AdcAII. Although both proteins deliver Zn(II) to the AdcCB transporter, AdcAII has a greater role during initial infection and survival in response to Zn(II) starvation. Despite this, the molecular details of how AdcAII selectively acquires Zn(II) remain poorly understood.

Aims
To date, our understanding of the Zn(II)-binding mechanism has been based solely on the crystal structure of Zn(II)-bound AdcAII, with an open, metal-free conformation remaining refractory to crystallographic approaches. As a consequence, the conformational changes that occur within AdcAII upon Zn(II)-binding remain unknown. This study aims to elucidate the molecular mechanism of Zn(II)-binding by AdcAII, and to determine the biophysical basis by which the protein achieves specificity for Zn(II).

Methods
Here, we overcame the lack of structural information by individually mutating each of the four Zn(II)-coordinating residues, and performing structural and biochemical analyses on the variant isoforms. X-ray crystallography and molecular dynamics simulations identified structural rearrangements that occur upon Zn(II) binding. Quantitative metal-binding experiments and phenotypic assays were further utilised to determine the contribution of each residue to ligand-induced conformational changes.

Results
Structural analyses revealed specific regions within the protein that underwent conformational changes via their direct coupling to each of the metal-binding residues. Biochemical and microbiological analyses further revealed that two of the four coordinating residues had essential contributions to the Zn(II)-binding mechanism of AdcAII. Intriguingly, only one of these residues had a direct role in structural rearrangements within AdcAII. These analyses also revealed that AdcAII could interact with other first-row transition metal ions, in contrast to AdcA and other Zn(II)-binding SBPs.

Conclusions
Collectively, our structural, biophysical and microbiological data indicate that AdcAII employs a distinct mechanism of metal binding to other Zn(II)-specific SBPs. Elucidation of this mechanism of Zn(II) uptake by *S. pneumoniae* will provide the structural and biochemical data required for future antimicrobial design strategies.

P-13 UNDERSTANDING THE IN VIVO FUNCTION OF A-TO-I RNA EDITING BY ADARs

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Introduction

Adenosine to inosine (A-to-I) editing is one of the most prevalent forms of post-transcriptional modification of RNA. Inosine (I) is interpreted as Guanosine (G) by both the ribosome and when sequenced, therefore, A-to-I RNA editing sites can be found as A-to-G mismatches between the RNA sequence and DNA sequence. A-to-I editing is mediated by the Adenosine Deaminase Acting on RNA (ADAR) protein families. In mammals, there are three ADAR enzymes: ADAR1 (ADAR), ADAR2 (ADARB1) and ADAR3 (ADARB2). Only ADAR1 and ADAR2 have A-to-I editing abilities. ADAR1 is expressed all over the body, while ADAR2 is mainly expressed in the central nervous system.

Most RNA editing happens in repetitive regions, potentially preventing the formation of paired double-strand RNA (dsRNA). Endogenous paired long dsRNAs can be detected by MDA5 (encode by the gene Ifih1) and regarded as foreign, resulting in the activation of the innate immune system in both human and mouse. One key physiological function of A-to-I editing, particularly by ADAR1, is to mark endogenous dsRNA to prevent it from being sensed as foreign.

However, other functions of ADAR1 are little understood. The detailed consequences of a loss of all A-to-I editing on the mammalian transcriptome are still largely unknown. This is due to no systems that completely lack A-to-I editing being established and characterized.

Methods

In order to understand the transcriptome-wide functions of RNA editing by ADAR1, our laboratory has been using genetically modified mouse lines that lack specific ADAR proteins or functions. The immortalized myeloid cell lines (a type of blood cell) was generated from Adar1+/+Ifih1-/- (WT) and Adar1E861A/E861AIfih1-/- (E861A). The Adar1 E861A mutation means Adar1 can no longer edit RNA. These cell lines do not express any Adar2 or Adar3 transcript, and so provide a completely A-to-I editing deficient and control genotype to understand how A-to-I editing regulates the transcriptome.

We take advantages of next-generation sequencing (NGS) and RNA-seq technique to profile the landscape of A-to-I editing from a genome-wide scale.

Results

We found RNA editing can cause gene differential expression between WT and E861A mice. For editing sites in a particular region, some of them are more likely to be edited than others. Also, these sites show a strong relationship with each other. They may occur together or be mutually exclusive. These correlations may be determined by the RNA secondary structure and specific sequence context.

P-14 ANTI-METASTASIS THERAPY VIA NANOPARTICLE MEDIATED DRUG DELIVERY

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Introduction and Aims
Metastatic disease is the major cause of cancer-related death in patients with solid tumours such as breast cancer. The mainstay of current treatments for metastatic disease is chemotherapy that is limited by systemic toxicity. To improve drug efficacy, there is a need for a mechanism to target tumour cells with minimal damage to normal tissues. Preliminary data show that the Unfolded Protein Response (UPR) chaperone glucose-regulated protein 78 (GRP78) is overexpressed on the cell surface of multiple metastatic breast tumour tissues. We have demonstrated that nanoparticles (NPs) can be targeted to colorectal cancer cells and doxorubicin-loaded NPs exhibited similar toxicity to treatment with doxorubicin alone in *in vitro* studies with SY5Y neuroblastoma cancer cells.

Methods
My project comprises the development and test of a tumour-specific drug delivery system using NPs that selectively target metastatic tumour cells by virtue of their high levels of surface-localised GRP78. NPs with optimal size and binding affinities for selective uptake by tumour cells will be analysed in terms of biodistribution, toxicity, half-life and efficacy in preclinical models of metastatic cancer.

Results
To date, a library of NPs loaded with Cyanine5 (Cy5) has been generated and characterised. Poly(ethylene glycol) (PEG)-ylated NPs equipped with a GRP78 binding peptide showed stronger tumour cell association with surface GRP78-positive murine 4T1.2 cells than NPs linked to a scrambled peptide or a PEG side chain alone. Replacement of Cy5 with doxorubicin or an apoptosis-inducing peptide will allow us to test cytotoxic properties of the NPs towards surface GRP78-positive breast cancer cell lines. Furthermore, phage display technology is used to identify stronger binding moieties to GRP78 to tune targeting efficiency of NPs.

Conclusions
These data reveal that NPs can be targeted to breast cancer cells and represent a promising approach for localised therapy of both primary and metastatic breast cancers.

P-15 CHARACTERISATION OF MANUALLY AGGREGATED DERMAL PAPILLA CELLS

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Introduction
Dermal papilla cells (DPCs) are responsible for controlling the hair cycle. These cells also have the ability to regenerate new hair follicles. This ability to regenerate hair follicles, or dermal papillae (DP), correlates with the aggregative characteristic of DPCs. *In vitro*, DPCs lose their aggregative behavior during cell expansion, and hence their ability to induce hair follicles after transplantation. Manual or forced aggregation of late passaged DPCs may be a way of generating viable, follicle forming DPs.

Aim
To generate and characterise manually aggregated DPCs with the aim of inducing the formation of follicle competent aggregates.

Methods
DPCs were manually aggregated in Terasaki plates with approximately 20,000 DPCs seeded per well. Immunofluorescence studies were performed using DPC markers, such as alkaline phosphatase, corin, and CD133/prominin-1.

Result
One day after the cells were seeded to Terasaki well, the DPCs demonstrated aggregate formation. The induced aggregates were then characterized by immunofluorescence to assess DPC-related markers.

Conclusion
Late passaged DPC can be manually induced to form aggregates that potentially have the ability to generate *de novo* hair follicles.
Significance: Aggregated DPCs may be transplanted in patients with baldness to promote hair regeneration.

P-16 CARTILAGE REPAIR USING RAPIDLY ISOLATED MESENCHYMAL STEM CELLS AND BIOFABRICATION TECHNIQUES IN ONE SURGICAL SETTING

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Introduction
Biofabrication is a strategy addressing cartilage repair by using bioscaffolds typically composed of materials and stem cells. A major barrier to clinical translation is the need for laboratory-based cell expansion and culture which leads to concerns with the use of two surgeries, increased recovery time, the use of animal serum-based media and sterility.

Aims
To establish a technique for cartilage repair using a pure stem cell population and biofabrication within one surgery, this is achieved by:
a) Developing a rapid isolation protocol of human Adipose-Derived Mesenchymal Stem Cells (hADSCs) from the Infrapatellar Fat Pad (IFP)
b) Identifying the minimal concentration of hADSCs laden in a GelatinMethacrylol/Hyaluronic Acid (GelMa/HA) hydrogel required to produce neocartilage
Finally, from these two aims, we can calculate if clinically significant cartilage defect sizes can be treated using this approach.

Methods
IFP was opportunistically harvested and processed using either our newly developed rapid (85 minutes) or the standard (>27-hour) isolation protocol. Cell retrieval assessment, immunophenotyping and a 3-week chondrogenic assay was performed. Next, $1cm^2$ bioscaffolds were fabricated using three hADSCs concentrations (representing 12.5, 25 and 50% of the healthy chondrocyte concentration) laden in a GelMa/HA hydrogel, after 3 weeks of chondrogenic stimulation a chondrogenic assay, immunostaining and imaging was performed.

Results
The rapid isolation approach resulted in a cell retrieval ability comparable to the standard protocol. Flow cytometry showed comparable profiles in both groups consistent with the hADSC phenotype and chondrogenic differentiation was comparable in both rapid and standard groups after 3-weeks as evidenced by GAG/DNA quantification and chondrogenic gene expression. The GAG content, chondrogenic gene expression and ECM accumulation of collagen 2 is highest in the 5.0 million hADSC/ml concentration bioscaffold group (50% of the healthy chondrocyte concentration).
Correlating data from aims 1 and 2 we identify that cartilage lesions up to 224 µl (224 mm^3) in volume can be repaired in one surgical operation using the minimum concentration and the number of rapidly isolated non-expanded hADSCs population.

Conclusions
These novel findings pave way for future progression to *in vivo* study of single-surgery cartilage repair using a pure stem cell population and biofabrication.

P-17 IMAGING POROUS CHANNELS IN THE OSTEOCHONDRAL INTERFACE: INVESTIGATING A NANOPARTICLE-BASED CONTRAST AGENT

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Introduction

Imaging porous channels in the osteochondral interface with micro-computed tomography (microCT) will provide a means to track joint nutrition and study joint health. However, it is necessary to use a contrast agent to better delineate the porous channels of the osteochondral interface from other regions with similar X-ray attenuation.

It has been demonstrated that hybrid metal nanoparticles composed of Barium (Ba), Ytterbium (Yb) and Fluoride (F) can provide excellent clinical CT contrast at different energies (80 - 140 kVp).

In order to image porous channels in the osteochondral interface in small animals (50 - 500 µm), microCT is required since it provides higher resolution (5 - 160 µm) at lower energy (45 - 70 kVp).

Aims

To test a hybrid nanoparticle-based contrast agent previously developed for clinical CT in microCT.

Methods

$BaYbF_5$ nanoparticles were synthesized through a series of thermal treatments (110°C and 300°C) as described in the literature. $BaYbF_5$ were coated with silica through a water-in-oil microemulsion method using TEOS as silica precursor. Silica nanoparticles (SiO_2) were also synthesized using the same method. Nanoparticles were imaged with Transmission Electron Microscopy, TEM (Tecnai F20, FEI, Oregon, USA) at 200 kV. Nanoparticle solutions with different concentrations (150, 200 and 250 mg/mL) were scanned by microCT (vivaCT80, Scanco Medical AG, Brüttisellen, CH) to obtain their X-ray attenuation profiles. Scans were performed with 10 µm voxel size; 70 kVp; 57 µA; 200 ms integration time.

Results and Conclusions

$BaYbF_5$ had an average diameter of 8 nm, as measured by TEM images. The hydrophobic $BaYbF_5$ nanoparticles were encapsulated with a silica shell creating an intrinsic aqueous solubility with a view to future experiments in small animals. TEM images showed that $BaYbF_5$-SiO_2 nanoparticles had a silica shell with an average thickness of 10 nm. The X-ray attenuation profiles of the nanoparticle solutions with three increasing concentrations exhibited increasing contrast in microCT.

$BaYbF_5$ nanoparticles were homogenous and monodisperse. They were successfully encapsulated with a silica shell becoming hydrophilic. For each concentration, $BaYbF_5$ nanoparticles exhibited higher CT value than either $BaYbF_5$-SiO_2 and SiO_2 nanoparticles. The three nanoparticle solutions at their highest concentration (250 mg/mL) had the highest x-ray attenuation. In order to improve the contrast further, the next step is to synthesize $BaYbF_5$-SiO_2 nanoparticles with a larger core and thinner silica shell. In conclusion, $BaYbF_5$ hybrid metal contrast agent is a good candidate for high-resolution imaging in microCT.

P-18 DEVELOPING A CORE OUTCOM SET TO MEASURE THE EFFECTS OF PAIN MANAGEMENT DURING LABOUR AND CHILDBIRTH

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Introduction
Labour can be one of the most painful experiences that a woman encounters in her lifetime. Pain management options available to women include pharmacological (such as epidural analgesia) and non-pharmacological interventions (such as massage and heat packs). These interventions can provide pain relief; however, trials of these interventions have used a wide variety of outcomes, complicating comparison of their effects. A core outcome set is a pre-defined list of required outcomes for conducting research on an intervention. Currently, there is no agreed core outcome set for the measurement of effects of pain management in labour and childbirth.

Aim
To develop a core outcome set for the management of pain during labour and childbirth.

Methods
We searched the Cochrane database (CENTRAL) for relevant systematic reviews of labour and childbirth pain management interventions. All outcomes reported within these systematic reviews (at review and trial level) were extracted and reported descriptively.

Results
We identified and extracted outcomes for 20 systematic reviews and 410 trials. Extracted outcomes were organized by importance (primary, secondary), participant (woman, infant) and type (benefit, harm, health service outcomes). Most frequent outcomes were identified for potential inclusion in a core outcome set.

Conclusion
Pain management options have varying effects on women and their newborns. The development of a core outcome set will provide future clinicians with a standardised method of recording and comparing data.

P-19 USING RADIOLABELED NEUROTROPHIN TO EXAMINE COCHLEAR PHARMACOKINETICS

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Introduction
Hearing loss is a prevalent condition with almost half a billion people worldwide having a significant hearing deficit. Local neurotrophin treatment has shown therapeutic potential in the protection and repair of cochlear sensory structure in hearing-loss models. However, pharmacokinetic studies of delivered neurotrophin in the cochlea remain challenging. As the cochlea has limited fluid volume and size, drug visualization and quantification using traditional techniques such as functional imaging and sampling are difficult to achieve. A more sensitive and accurate approach is needed in order to reveal details of drug distribution, retention, and target-tissue uptake.

Aim
To develop a method for intracochlear radiolabel-based analysis at histological and cellular levels under cochlear-fluid volume and component control.

Methods
Guinea-pig cochleae were treated with an acute-injection and a slow-release system delivering 125I-labeled neurotrophin-3. At 4-hour time point, animal cochleae with sealed surgical openings were harvested for whole-cochlear gamma counts. Cross-sections were obtained and exposed to emulsion in film and in emulsion-coated slides for autoradiography. Visualized section-based and tissue-based radiolabel signals were imaged and quantified via ImageJ software by drawing regions of interest for densitometry.

Results
The radiolabeling technique was effective in analyzing neurotrophin distribution and clearance in the cochlea following delivery via a drug-injection cannula or via a slow-release system. 125I-neurotrophin-3 was detected throughout the entire cochlea and the target tissues at different locations for both delivery systems. Gamma count results were able to indicate the absolute 125I-neurotrophin-3 level in the entire organ (cochlear fluids and tissue together). Quantification results showed diverse 125I-neurotrophin-3 levels at different regions of the cochlear sections. At 100 X magnification, quantification results at the cochlear basal region also showed diverse 125I-neurtrophin-3 uptakes across several types of tissue, including the sensory tissue, primary auditory neurons and their peripheral fibers.

Conclusion
Whole-cochlear gamma counts, radiolabel-signal visualization through film- and slide-based autoradiography, and quantification on radiographic images together can generate high-resolution results comprising multiple cochlear pharmacokinetic patterns and parameters. Methods employed to visualize and quantify radiolabel signal in this study can be repeatedly used to acquire high-quality pharmacokinetic results in other intracochlear drug-delivery strategy studies. The same methods may also apply to pharmacokinetic studies of drug in other small biological systems.

P-20 DETERMINING THE ROLE OF MATERNAL EPIGENETIC INHERITANCE IN BONE DEVELOPMENT AND DISEASE

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Environmental influences such as diet, drugs and chemicals are thought to alter epigenetic programming in oocytes and contribute to maternally inherited disease. Exposure of the developing germline to these lifestyle factors can provoke epigenetic alterations in gametes, and thus modify offspring development and phenotype. Embryonic Ectoderm Development (EED) is one of the interdependent subunits of the epigenetic modifier Polycomb Repressive Complex 2 (PRC2), which mediates epigenetic reprogramming in oocytes that is essential for offspring development. PRC2 catalyses the tri-methylation of lysine 27 in histone 3 (H3K27me3) and is enriched in the promoter regions of genes that are developmentally important. Many of these genes play important roles in stem cell differentiation in fetal and adult tissues, including in developing bone, the hematopoietic system and the brain. Whilst the role of EED in regulating a range of stem cell populations in fetal and adult tissues is well understood, the role of PRC2 in epigenetic programming of the egg and sperm and its consequent influence on inherited developmental characteristics and offspring health is poorly understood. In humans, *de novo* germline mutations in *EED* result in Cohen-Gibson syndrome, which is characterized by fetal overgrowth, accelerated bone aging and skeletal defects. Moreover, we recently established a mouse model in which *Eed* is specifically deleted in growing oocytes, resulting in eggs that lack EED-dependent epigenetic programming. Heterozygous offspring produced from these eggs were characterised by overgrowth and altered bone density compared to genetically identical heterozygous controls, characteristics reminiscent of Cohen-Gibson syndrome in humans. Using this model, the current study aims to determine how EED-dependent programming in oocytes regulates bone development, skeletal phenotype and bone repair in offspring. The outcomes will assist in identifying how inherited epigenetic information controls both early life and long-term developmental outcomes, which is crucial for understanding how epigenetic mechanisms impact the developmental origins of disease.

P-21 AUTOCRINE LAMININ-511 IN TRIPLE NEGATIVE BREAST CANCER IMMUNOBIOLOGY

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Introduction

Triple negative breast cancer (TNBC) is aggressive and has a high propensity to metastasise. Currently, there is no effective targeted therapy for TNBC, and most patients will develop resistance to chemotherapy, the conventional option available. Immunotherapy may provide an approach to control this disease. However, breast cancer has poor immunogenicity, in part due to impaired expression and presentation of MHC class-1, and expression of programmed cell death -1 ligand (PD-L1) and limited immune infiltration.

Accumulating evidence suggests that the matrix composition of breast tumour and the repertoire of surface adhesion receptors of cancer cells may play a role in immunogenic potency. Previous studies have shown that the extracellular matrix protein laminin 511 (LM-511) is produced abundantly in TNBC and is involved in metastatic progression. However, its role in immune-surveillance and immune-infiltration has yet to be determined.

Results

Herein, we performed an immunohistochemical analysis of a syngeneic model of TNBC (4T1BM2) and demonstrate that genetic suppression of LM-511 results in significantly increased infiltration of CD8+ and CD4+ T cells into the mammary tumour. A trend towards increased infiltration of MPO^{+ve} neutrophils, $F4/80^{+ve}$ macrophages and $Foxp3^{+ve}$ regulatory T cells was also observed. Using recombinant LM-511 and a unique laminin-511-binding antibody developed by our group, we provide proof of principle that secreted LM-511 may bind the surface of circulating tumour cells and increase their survival and proliferative potential *in vitro.* Lastly, we provide evidence that the presence of LM-511 on the surface of tumour cells may shield and partially protects them from immune cell killing.

P-22 IS OBESITY AS AN EARLY SIGN OF CREUTZFELDT–JAKOB DISEASE?

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Introduction

The existence of prion strains thought to be encoded by different conformations of the misfolded prion protein, pose a challenge to the diagnosis, treatment and prevention of human prion diseases. Despite their common pathology human prion strains have different clinical presentations that affect cognitive, motor and sensory functions. This variability can confound medical diagnosis, delay effective treatment and could lead to unexpected disease transmission. This is further hampered by the lack of medically relevant human-derived prion models for the development of effective treatment and prevention.

Recently we generated a human-derived mouse adapted strain of prion disease, MU-03, isolated from a patient exhibiting the most common form of human prion disease, sporadic Creutzfeldt–Jakob disease (Ethics no. 0707227, 1111949 and 1413151). This prion strain unusually exhibits weight gain as the first disease sign in the mice. While obesity is not considered a sign of prion disease in humans there have been multiple published descriptions of CJD patients who have struggled with weight gain and/or overeating. The recapitulation of this patient experience in our mice lead us to commence examination of the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) patient database to determine if obesity could be a phenotype of human prion disease (Ethics no. 1341074 and 1647293). There were significantly more CJD patients with a past history of obesity or who struggled with weight gain and/or over-eating compared with non-CJD patients suffering from other neurological illnesses.

Rapid obesity of an unknown origin could be an indication of prion disease in a subset of patients. Furthermore, the presence of this sign following adaptation in mice further supports the use of this prion strain for developing effective methods for the prevention and treatment of medically relevant human prion disease.

P-23 UNDERSTANDING INNATE IMMUNE DEFENCE MECHANISMS AGAINST THE INTRACELLULAR BACTERIUM *SALMONELLA* TYPHIMURIUM

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Introduction
Intracellular bacterial infections remain a major problem in human health. Although pathogen recognition and programmed cell death (i.e. pyroptosis, necroptosis, apoptosis) is thought to play an important role in controlling these pathogens, defects in individual pathways of pathogen recognition and programmed cell death often only cause minor defects in the *in vivo* control of such infections. This is likely related to redundancy that evolved in response to pathogen evasion strategies, which allows the host to compensate for evasion of one pathway through the activation of another.

Aims
Detailed knowledge about the *in vivo* organisation and regulation of the compensatory utilization of distinct pathways during intracellular infections is still missing. Furthermore, host and bacterial factors driving programmed cell death need to be identified and investigated.

Methods
An *in vitro* bacterial infection assay using bone marrow-derived macrophages was established to measure bacterial burden and cell death caused by *Salmonella* Typhimurium. Additionally, different strains of knock out mice for adaptors in pattern recognition pathways were infected with *Salmonella* Typhimurium *in vivo* to analyse the host control of intracellular infections.

Results
The successful establishment of an *in vitro* bacterial infection assay lead to the measurement of the correlation of bacterial burden and cell death caused by *Salmonella* Typhimurium. This assay will allow the analysis of molecules involved in pathogen recognition and cell death pathways. Interestingly, elevated bacterial titres were observed in TIR-domain-containing adapter-inducing interferon-beta (TRIF)-deficient mice following *in vivo* *Salmonella* Typhimurium infection, but not in other pattern recognition pathway knock out mouse strains.

Conclusions
The results of the *in vitro* bacterial assay suggest that intracellular replication is prevented by programmed cell death of host cells. The resulting removal of the replicative niche re-exposes the pathogen to extracellular mediators of the immune system, thus facilitating their detection, attack and clearance. The *in vivo* experiments suggest an important role for TRIF in the clearance of intracellular bacterial infections and a possible role for this molecule in cell death regulation.

P-24 COUPLING OF RESTING STATE NETWORKS IN ADOLESCENT PROBLEMATIC SUBSTANCE USE AND DEPRESSION

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Introduction
Emotion regulation (ER) deficits are apparent in both depression and substance use problems, both of which are prevalent during adolescence. Alterations in ER neural circuitry (specifically, altered connectivity between subcortical [e.g., amygdala] and frontal regions) have been implicated in both depression and substance use problems, and several neurodevelopmental models suggest that they may represent a common neurobiological marker. Substantial research has shown that activation of isolated regions and/or functional connectivity between specific regions is altered in youth depression and substance use; however, no research has investigated both types of mental health problems in the same study using system-level network approaches to investigate functional connectivity.

Aim
The primary goal of the study was to examine if inter-network coupling between the subcortical network and the executive control network was different for adolescents with MDD and/or problematic substance use.

Methods
We used resting-state functional magnetic resonance imaging (fMRI) and independent component analysis (ICA), a data-driven approach, to delineate cortical and subcortical functional networks in 108 adolescents (mean age = 17.59 years, SD = 1.22 years, 58 females) from the community. Dual regression was then used to obtain subject-specific time series for each network. We examined the relationship between coupling of the ECN and SCN and both depression (major depressive disorder [MDD] diagnosis) and problematic substance use (based on the youth risk behaviour survey [YRBS]).

Results
We found problematic substance use to be associated with coupling of the ECN and SCN. Problematic substance users showed decreased (more negative) coupling compared to non-problematic/non-users. We did not find MDD-related alterations in the coupling of these networks. However, exploratory analyses revealed alterations in coupling of the ECN and default mode network (DMN) in adolescents with MDD.

Conclusion
An altered relationship between the subcortical and prefrontal cortical systems may underlie ER deficits in adolescent problematic substance use, but not depression. We found greater negative coupling between the SCN and ECN in adolescent problematic substance users. Greater negative coupling could indicate a failure to bring cognitive control regions online in response to negative affect--contributing to the onset of early substance use. We also found lower connectivity between the DMN and ECN in depressed adolescents. The DMN is responsible for self-referential processing, therefore altered connectivity between the DMN and CEN could speak to depression-associated propensity to ruminate. Further work is needed to understand the causal nature of associations.

P-25 BONE AUTOMATED SEGMENTATION AND INTERACTIVE ANALYSIS OF LENGTH IN µCT SCANS (BASILISC)

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Introduction
Analysis of skeletal components of model organisms is essential for the understanding of limb development and repair. Differences between samples can be quantified using limb bone length as a parameter for comparison. Current approaches are very labor-intensive, as they require careful dissection, skeletal preparation, flat mounting and imaging, and manual measurement. Alternatives include microCT (µCT) scans for visualization of skeletal components, followed by 3D modelling and analysis. Nonetheless, these techniques require heavy user interaction, thus making them time-consuming.

Aims
We propose standardized µCT scanning, followed by image processing with Mimics software which also allows for data exporting, facilitating subsequent analysis. BASILISC aims to reduce current analysis time and creates a pipeline for semi-automated batch processing.

Methods
The pipeline consists of acquiring µCT data as DICOM files, followed by segmentation of long bones of the limbs, and measurement of each element. These steps have been optimized, ensuring the orientation of the samples and scanning parameters are standardized, which allows for a common threshold range across different samples, for ease of automation through Python scripting. Once segmentation has taken place, a center-line is fitted to each element, and the length of all the lines is exported within a comma-separated-values file. The developed approach is semi-automatic, where limited user interaction is required, reducing significantly the time taken from sample collection to bone measurement. Another advantage of using DICOM files is that metadata is preserved, including scale information.

Results
As proof of concept, BASILISC has been used to analyze mice with left-right asymmetry, at 2 different developmental stages (17.5-day embryos and 7-day post-natal). Results have demonstrated consistent absolute measurements across batches (scanning at different days and at high and low resolution- 20µm and 40µm respectively). Dependence on threshold and resolution has been observed for hind-limbs when analyzing an internally consistent ratio (left/right length), these data have been used to further optimize analysis conditions. In future, samples will be scanned at a low resolution and the pre-selected age dependent threshold.

Conclusion
In conclusion, BASILISC is a novel pipeline to measure the bone length with high throughput and minimal user interaction. It was proven to reduce analysis time 10-fold (~40hrs to ~4hrs when compared to current standard techniques), it's flexible as it can be applied to different developmental stages and can be adapted to other bone elements (i.e. analysis of intervertebral distance). Moreover, CT analysis is a non-destructive approach which will allow for maximization of tissue used for other experiments.

P-26 STRUCTURAL CONNECTOME FINGERPRINTING

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Introduction
Connectome fingerprinting, the process of identification of individuals from their brain activity data, has recently garnered significant attention. It has been shown that functional magnetic resonance imaging (fMRI) recordings of individuals carry unique information that not only acts as an identifier but can also reflect cognitive abilities such as fluid intelligence. Although substantial research regarding fingerprinting has been conducted using fMRI, the extant literature using white-matter tractography does not adequately leverage structural connectivity for the purpose of fingerprinting.
On the other hand, current research has not compared the identification capabilities of the functional fingerprint relative to identification using anatomical information.

Aims
The objective of this research is to first introduce a new method of brain fingerprinting based on structural connectivity patterns and to evaluate the differences of various fingerprinting features in the identification of individuals.

Methods
The openly available data from the human connectome project was used to collect anatomical information (such as cortical thickness and curvature), functional connectivity, and structural connectivity of 1000 subjects. For 42 subjects, a second retest scan from the same dataset was collected to be used in the identification task. For each feature, a similarity metric was computed to measure the similarity of two different instances of that feature. The similarity measure was then used to identify the retest subjects in a pool of all 1000 subjects.

Results
We found that some anatomical features, such as curvature of the cortical surface, performed significantly better than the functional fingerprint in the identification of the subjects. However, the structural fingerprint had the best performance in subject identification.

Conclusions
We showed that the new method of structural fingerprinting provided a better measure for an identification task. On the other hand, it was shown that identification by itself does not necessarily make a fingerprinting measure informative as an interpretable predictor of cognition/behaviour, as features like curvature perform well for the identification task. Therefore, further study on tasks to evaluate the interpretability of these fingerprinting measures using behavioural data is essential.

P-27 OPTIMISING A NEW VENTANA MMR IHC PANEL PROTOCOL FOR USE IN DIAGNOSIS OF COLON CANCER AT BOX HILL HOSPITAL

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Introduction

The Box Hill Hospital Anatomical Pathology laboratory routinely uses the Ventana Mismatch Repair (MMR) Immunohistochemistry Panel to distinguish between sporadic colorectal cancer and hereditary colon cancer. Loss of staining in any of the MMR panel antibodies (MLH-1, MSH-2, MSH-6 and PMS2) suggest a diagnosis of Lynch Syndrome, a hereditary non-polyposis colorectal cancer. Ascertaining this from a patient sample is of high importance as for a positive test for Lynch Syndrome means that all family members must be tested as well. There have been numerous issues with the antibody kit used in the laboratory and a move is being made to begin using a "ready-to-use" pre-diluted antibody kit over the current kit which involves diluting the antibodies for each use. Constantly repeating the panel because of issues with staining is delaying diagnosis and treatment for patients.

Aims

The aim of this project is to optimise an appropriate protocol for the new ready-to-use MMR antibody panel and to compare and contrast with the method currently employed in the laboratory.

Methods

This experiment will utilise Ventana Benchmark II Immunohistochemistry autostainers, trialling a variety of antibody retrieval times, antibody concentrations, incubation times and temperature settings to ascertain the best protocol for optimum staining. A variety of normal tissue, tissue with sporadic colon cancer and tissue with hereditary colon cancer will be tested to examine the efficacy of the new protocol. It is hypothesized that the new ready-to-use kit protocol will give better quality staining than the self-diluted method currently used, leading to a lower turnaround time on results and faster treatment for patients.

P-28 THE BAFF/APRIL SYSTEM IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Introduction
CLL is a B cell malignancy characterised by an accumulation of CD5+, CD19+ B cells in the blood and secondary lymphoid organs.

B cell-activating factor of the tumour necrosis factor (TNF) family (BAFF) and a proliferation-inducing ligand (APRIL) belong to the TNF superfamily and are important in normal B cell maturation and function. BAFF ligates with the receptors B cell receptors BAFF receptor (BAFFR), transmembrane activator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA), while APRIL signals through the receptors TACI and BCMA.

Previous research has revealed that the classical NFKappaB pathway is particularly important for CLL cell survival. While BAFF and APRIL signal through BAFFR to activate the alternative NFKappaB pathway, they stimulate TACI and BCMA to activate the classical NFKappaB pathway. BAFF and APRIL are produced by CLL cells and nurselike cells in the CLL microenvironment and promote CLL cell survival.

Aims
The aims of this project are to examine the effects of stimulants on TACI and BAFFR expression on CLL cells and to analyse the role of TACI and BAFFR in CLL signalling. Understanding how CLL signalling occurs will enable us to potentially target these signalling pathways to halt CLL progression.

Methods
We derived our cells from the TCL1-Transgenic (TCL1-Tg) mouse model which is a CLL mouse model. These cells were exposed to various stimulants through in vitro culturing and short stimulation to understand CLL signalling.

Results
Findings from my project illustrate that CLL cells have higher TACI and BAFFR surface expression, and survive and proliferate better when stimulated with LPS compared to other stimulants such as anti-mu, CpG and CD40L. Normal B cells proliferate better compared to CLL cells when co-cultured with CD40L or LPS. The addition of anti-mu to LPS or CD40L increases the Fas surface expression. These cells have lower TACI and higher BAFFR surface expression compared to the other CLL cells.

Signalling analyses have elucidated that BAFFR may be a negative regulator of the activation of the MAPK pathway following BAFF and APRIL stimulation. BAFF and APRIL result in the increase of pro-apoptotic proteins FLIP and mcl-1.

Conclusion
These findings indicate that the receptors including the BAFF/APRIL receptors and signalling pathways of CLL cells can be modulated by the stimulants in the microenvironment which may render them more conducive to survival or apoptosis. Understanding how stimulants shape CLL cells will enable us to devise ways to manipulate these signals to limit CLL progression.

P-29 INVESTIGATING THE ROLE OF SHORT CHAIN FATTY ACIDS IN CD8 T CELL IMMUNITY

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Introduction

CD8 T cell fate decisions are governed by multiple cues that occur during an immune response. These factors determine the balance between memory precursor and short-lived effector T cells (MPECs and SLECs respectively). Change in metabolic control is one such cue. Distinct functions of memory and effector T cells dictate different metabolic requirements. Memory T cells perform more fatty acid oxidation and oxidative phosphorylation than effectors. Factors promoting these processes include short chain fatty acids (SCFAs). Preliminary findings highlighted that the SCFA butyrate promotes MPEC formation. However, underlying mechanisms of how butyrate does so is still poorly characterised. SCFAs are endogenous ligands for the orphan G protein-coupled receptors 41 and 43 (GPR41/43), and may hence mediate CD8 T cell differentiation through these receptors.

Aims

We aim to analyse whether SCFAs influence T cell fate decisions and effector functions by promoting the development of memory CD8 T cells. Mechanisms by which they do so will also be elucidated.

Methods

Ffar2$^{-/-}$*;Ffar3*$^{-/-}$ (encoding for GPR43 and GPR41 respectively) mice were epicutaneously infected with HSV-1, and endogenous CD8 T cell responses analysed by Kb-glycoprotein $B_{498\text{-}505}$ ($gB_{498\text{-}505}$) tetramer staining one week post-infection. *In vitro* activated gBT-I (transgenic CD8 T cells that express a T cell receptor specific for the immunodominant peptide $gB_{498\text{-}505}$ from Herpes Simplex Virus (HSV)) were incubated with different concentrations of the SCFAs acetate, propionate or butyrate for 2 days. The treated gBT-I cells were then adoptively transferred into naïve recipient mice, that were infected with HSV on day 14 post-transfer. CD8 T cell differentiation and cytokine production of the gBT-I cells were analysed using flow cytometry.

Results

Adoptive transfer of SCFA-treated gBT-I cells indicated that butyrate promoted MPEC formation, while acetate and propionate had negligible effects following *in vivo* HSV challenge. Interestingly, frequencies of TNF alpha-expressing MPECs and granzyme B-expressing SLECs were increased following butyrate treatment. In contrast, HSV-specific cytotoxic T cells lacking GPR41 and GPR43 expression developed lower MPEC and higher SLEC frequencies compared to wild type mice infected with HSV.

Conclusions

These findings suggest that GPR41/43-mediated pathways influence CD8 T cell fate decisions and memory formation. Interestingly, while the SCFAs acetate, propionate, and butyrate can bind to these receptors, only butyrate had the potential to promote memory formation of CD8 T cells. Butyrate also enhanced effector function, with distinct differences in cytokine-producing effects between MPECs and SLECs. These findings suggest that SCFAs and their receptors influence CD8 T cell differentiation.

P-30 ANALYSIS OF INFLAMMATORY CELLS IN THE DIAGNOSIS OF SKIN CANCERS

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Introduction
The extremely subtle shift from inflammation to tumour is an area of great scientific interest. Although inflammation derived by environmental factors has a well-known underlying mechanism, carcinogenic changes following a chronic inflammatory state that can lead to a tumour-promoting process in the tissue microenvironment is less known. The maintenance of the inflammatory microenvironment is conducive to tumorigenesis. Inflammation promotes the proliferation and survival of malignantly transformed cells that relate to angiogenesis and metastasis. Non-melanoma skin cancers, including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are frequently diagnosed types of cancer in Australia, which have significant inflammatory components. Histological examination of tumour markers is a gold standard in clinical diagnosis and can confirm the diagnosis made by a pathologist, however, our understanding of the presence of inflammatory cells in skin cancer and the potential to be in diagnosis is not known.

Aims
To analyse the frequency and intensity of inflammatory cell markers: CD1a, CD15, CD23, CD68 in previously diagnosed cases of skin cancers, including SCC, BCC and melanoma and investigate diagnostic and/or therapeutic potential.

Methods
Archival formalin fixed paraffin embedding (FFPE) blocks received by RMIT University, Pathology Unit were cut, examined using haematoxylin and eosin (H&E) stain to describe the microscopic details and immunohistochemistry (IHC) using the following antibodies: CD1a, CD15, CD23, CD68; to analyse the presence and distribution of inflammatory cells in response to skin cancers.

Results
Expression and intensity of expression of antibodies against inflammatory cell markers were analysed from various human skin cancer cases and results presented.

Conclusions
Analysis of inflammatory cell markers by IHC expression in human skin cancers may be used as a diagnostic tool and/or a mechanism to better understand skin cancer biology.

P-31 IMMUNOHISTOCHEMISTRY (IHC) PANEL FOR THE DIAGNOSIS AND DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA (SCC)

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Introduction
In Australia, approximately 400,000 cases of non-melanoma skin cancers (NMSC) are diagnosed per year. The primary cause of skin cancers is prolonged exposure to ultraviolet radiation, with increased risk in individuals with fair skin, predisposition for sunburn and previous skin cancer. In Australia, 70% of NMSC are basal cell carcinomas (BCCs), while 30% of NMSC are squamous cell carcinomas (SCCs). Compared to BCC, SCC lesions are fast-growing and can spread in the body if untreated. Therefore, the differentiation between SCC and BCC lesions are important for histological diagnosis to determine prognosis and treatment for a patient.

Aim
To design an IHC panel for the diagnosis and differentiation of SCC.

Method
The antibodies chosen for the IHC panel were EMA, BerEP4, CEA, CD10, S100 and BCL-2. Current antibodies available to diagnose SCC is by the elimination of BCC positive tumour markers. Selection of the antibodies was based on known tumour markers to confirm the diagnosis of SCC and eliminate the differential diagnoses of BCC and melanoma. To test the efficacy of the antibodies, a range of control tissues were used to determine stain quality and suitability for each antibody. The panel was then used to diagnose SCC in archival human skin cancer samples.

Results
The application of negative and positive controls is working appropriately. EMA and CEA staining confirmed the diagnosis of SCC in skin sections. CEA positivity was also observed in melanoma; however, SCC diagnosis was confirmed with negative S100 staining as melanoma is positive for S100. Negative staining in BerEP4, CD10 and BCL-2 ruled out the diagnosis of BCC, where these antibodies are positive.

Conclusion
The use of EMA in the IHC panel confirmed the diagnosis of SCC in skin sections. The inclusion of S100, BerEP4, CEA, CD10 and BCL-2 in the IHC panel ruled out BCC and melanoma. Confirmation of SCC diagnosis in this panel was by observing negative staining in these antibodies. However, the IHC panel used is limited in its basis on the absence of staining to diagnose SCC.

P-32 THE AUSTRALIAN GENOMICS AND INHERITED CANCER CONNECT HEREDITARY CANCER SYNDROME PROJECT

★ ***BML travel award***

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Introduction

Cancers may be driven by germline variants which are present in the normal DNA of a cancer patient. Many of these germline drivers are inherited, such that multiple individuals within families present with cancer phenotypes that are considered characteristic for a given gene or genes. Such presentations are termed hereditary cancer predisposition syndromes (HCPS). While multiple causative variants for these HCPS have been identified, the underlying causes for many HCPS remain unknown. We present a project targeting individuals with these unexplained HCPS is being conducted by Australian Genomics and the Inherited Cancer Connect (ICCon) partnership.

Aims

The Australian Genomics and ICCon project aims to analyse the germline whole-genome sequences of 190 patients presenting with clinical features of a HCPS who have previously undergone uninformative clinical genetic testing.

Methods

Our analysis includes the identification of germline variants, including single-nucleotide, small insertions and deletions, copy-number and structural events, within a reviewed gene panel. These genes have disease-associated variants which are considered clinically actionable within the context of familial cancer.

Results

To date, we have recruited 104 participants of which 68 have undergone whole-genome sequencing. Consequently, we have reviewed more than 600 rare variants for potential association with cancer. Approximately, 10% of these variants were discussed at multi-disciplinary team meetings, of which the majority were reported as variants of uncertain significance and several of likely pathogenicity. These findings have also triggered requests for functional assays to aid variant interpretation, and further detailed clinical examination to assess the relationship between identified variants and patient presentation.

Conclusions

We hope that identifying these variants will increase our understanding of the role that germline variation has in the hereditary susceptibility of cancer, and in doing so identify better ways to manage patients with otherwise unexplained hereditary cancer.

P-33 DETERMINING THE ROLE OF MATERNAL EPIGENETIC INHERITANCE IN OFFSPRING BEHAVIOUR AND BRAIN DEVELOPMENT

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Introduction
Epigenetic modifications, such as DNA methylation and covalent histone modifications, involve heritable changes in gene expression without changing the underlying DNA sequence. Significantly, activity of epigenetic modifying enzymes and the signatures they establish can be altered by environmental influences, such as diet, drugs, chemicals and stress. Exposure of the developing germline to these environmental factors can elicit epigenetic changes and contribute to inherited disease in offspring. Work in mice has shown that a specific epigenetic regulator, Polycomb Repressive Complex 2 (PRC2), is required in oocytes for growth and development in offspring. PRC2 catalyses tri-methylation of lysine 27 in histone 3 (H3K27me3), which inhibits gene expression. H3K27me3 is often found in promoter regions of developmentally important genes thereby repressing target genes and regulating cell differentiation in multiple tissues, including brain, bone and
germ cells. In humans, de novo germline mutations in the essential PRC2 encoding genes *Embryonic Ectoderm Development* (*EED*) or *Enhancer of Zeste 2* (*EZH2*) result in Cohen-Gibson and Weaver syndromes, respectively, which are characterised by fetal overgrowth, skeletal defects and reduced learning capacity in children.

Methods
We have developed a model that deletes EED only in growing oocytes in mice, enabling the study of epigenetic inheritance through the production of genetically identical offspring from eggs that have differences in EED-dependent epigenetic programming.

Results
This model has demonstrated that loss of EED in the female germline results in fetal overgrowth and skeletal defects, reminiscent of Cohen-Gibson Syndrome in humans. The current study aims to characterise the impacts of the loss of EED in mouse oocytes on offspring learning capacity, brain development and behaviour.

Conclusion
The outcomes will provide the first insights into the role of EED, and the epigenetic mechanisms it modulates, for regulating heritable changes in brain development and behaviour. Understanding this and similar epigenetic mechanisms are critical for determining how environmental factors in a parent, such as drug or dietary exposures, may affect learning and behavioural outcomes in offspring.

P-34 MODELLING ALZHEIMER'S IN PLURIPOTENT STEM CELLS USING CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEAT/CAS9

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Introduction
Alzheimer's disease (AD) is a neurodegenerative disease characterised by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular hyperphosphorylated Tau (pTau). AD has two forms, the familial form (fAD), and the sporadic form. fAD is caused by the autosomal inheritance of mutations in either presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) or amyloid precursor protein (*APP*). Literature suggests that retinal degeneration occurs prior to the onset of cognitive decline, therefore positioning the retina as a potential model for prodromal AD. The outermost layer of the retina, the retinal pigment epithelium (RPE), is responsible for normal photoreceptor maintenance and has been reported to exhibit AD-associated changes such as Aβ accumulation in animal and post-mortem studies. Despite this, the pathological mechanisms underlying the RPE's dysregulation and degradation in AD are poorly understood. Human-induced pluripotent stem cells (hiPSCs) are a powerful tool for disease modelling tool due to their ability to self-renew and differentiate into any adult cell type, including the RPE. In combination with gene-editing technology CRISPR-Cas9, researchers are now able to precisely edit genetic sequences to restore normal gene function in patient-derived iPSCs. In tandem with other models, hiPSC derived from AD patients may offer researchers the ability to further elucidate the AD phenotype implicated in the RPE.

Aims
To correct hiPSCs derived from AD patients with mutations in *PSEN1* using the CRISPR-Cas9 system and then differentiate into RPE to model AD *in vitro.*

Methods
Patient-derived *PSEN1* iPSCs were corrected for the H163R SNP using CRISPR-Cas9 homologues directed repair. These cell lines were screened and validated using flow cytometry, PCR and Sanger sequencing. The successfully corrected isogenic CRISPR-Cas9 clones and uncorrected *PSEN1* lines were differentiated into RPE using our established methodology. After 90 days of differentiation and maturation, cells were fixed and stained for hallmark markers of AD including Aβ using traditional immunohistological and cytochemical (IHC and ICC) approaches.

Results
We have successfully generated an isogenic control of the *PSEN1* mutation in iPSCs using CRISPR-Cas9, which have been well-characterised for pluripotency markers by ICC. Following this, we aim to establish a retinal model to study AD, where both isogenic control and diseased *PSEN1* lines will be differentiated to RPE for 90 days and screened for histological markers of AD including Aβ.

Conclusion
We have successfully corrected the *PSEN1* mutation in patient-derived iPSCs using CRISPR-Cas9, which will be used to create an *in vitro* model to study AD markers in the RPE.

P-35 INTESTINAL IMMUNE AND STROMAL CELL RESPONSES TO ACUTE AND CHRONIC VIRAL INFECTION

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Introduction
Lymphocytic Choriomeningitis Virus (LCMV) is routinely used as a model to study immune responses in the context of acute and chronic viral infection. In the gut, the majority of immune processes take place in the intestinal mucosa, where effector immune cells are distributed. While it is established that cytotoxic intra-epithelial lymphocytes (IELs) contribute to the elimination of LCMV-infected cells in the intestinal mucosa, the dynamics and roles of other immune cells in the gut (such as myeloid cell populations and ILCs) have not been fully characterised.
Additionally, stromal cells of the intestine can regulate inflammation, as they are able to sense and respond to environmental signals generated by injury or infection, and interact with immune cells directly and via the production of cytokines. While more is known about lymphoid organ stromal cell responses to LCMV infection, little is known about how the stromal cell compartment of the intestine responds to viral infection.

Aims
Firstly, we aimed to characterise and compare the kinetics of LCMV-specific T cells, endogenous myeloid and innate lymphoid cell populations in the intestine during acute and chronic LCMV infection. Secondly, we aimed to identify intestinal stromal cell subsets, and characterise how they respond to acute and chronic LCMV infection.

Methods
To do this, immune and stromal cell subsets from the small and large intestines of mice infected with an acute or chronic LCMV strain were analysed by FACS and confocal microscopy at different times post-infection.

Results
At the peak of the response to acute infection, there are similar numbers of LCMV-specific CD8+ T cells in acutely and chronically infected mice, however, cytokine responses are lower in chronically infected mice. During chronic infection, the large intestine has an increased number of LCMV-specific CD8+ T cells which is not seen in the small intestine.
The frequency of LCMV-specific CD8+ memory T cells co-expressing CD69 and CD103 in the large intestine differs to that in the small intestine after acute infection. Intestinal ILC, myeloid and stromal cells decrease in number in chronically infected mice compared to naïve and acutely infected mice. Macrophages and stromal cells are infected more by the chronic LCMV strain compared to the acute strain, and this could contribute to their decrease in number.

Conclusion
Persistent viral infections represent important global health problems. These studies will contribute to a greater understanding of how stromal cells may regulate immune responses in the intestine during chronic viral infection.

P-36 DEVELOPING AN IMMUNOHISTOCHEMISTRY (IHC) PANEL FOR THE DIAGNOSIS AND DIFFERENTIATION OF MELANOMA

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Introduction
Melanoma is a type of skin cancer which is caused by malignant transformation of melanocytes. Melanoma is considered to be one of the most common cancers to be diagnosed in Australia and New Zealand. One of the risk factors of melanoma is from the exposure of the sun. The survival rate of melanoma would decrease as the stage of the disease increases. Immunohistochemistry (IHC) is considered to be an important role in diagnosing skin cancer and is easier applied when comparing to other molecular methods.

Aims
This research aims to confirm the diagnosis of melanoma with the application and development of an IHC panel.

Methods
Fifteen formalin-fixed paraffin-embedded (FFPE) patient samples were received and sectioned for Haematoxylin and Eosin (H&E) staining in addition to an IHC panel. The tissue sections were examined accordingly to confirm and determine the probable diagnosis. The confirmation was initially conducted with the use of various control blocks to which provided validated staining. IHC markers such as s100, Melan A, HMB45 and CD34 were applied in the panel to identify melanoma. The test sections were carried out using the IHC panel and analysed through light microscopy. Stained slides were analysed and examined with a scoring rate based on the staining intensity.

Results
The test sections that were presumed melanoma were stained positive for the included melanoma-specific markers, opposed to the rest of the cases that were presenting with a skin cancer other than melanoma, which presented with negative results.

Conclusion
Through the analysis of s100, Melan A, HMB45, and CD34 expression, these IHC markers have demonstrated the capability in diagnosing and differentiating melanoma.

P-37 DEVELOPING A DOUBLE-LABEL IMMUNOHISTOCHEMISTRY (IHC) PANEL FOR THE DIAGNOSIS OF SKIN CANCERS (IN HOUSE KIT)

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Introduction

Skin cancer has three common sub-types: melanoma and two non-melanoma skin cancers, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Immunohistochemistry (IHC) is a useful tool in the differentiation and diagnosis of the various types of tumours arising from skin cells or present on the skin. IHC makes it possible to locate target antigens by using antibodies that bind to the specific antigens. Chromogens are then applied to allow accurate visual representation of their presence. Double staining is an immunohistochemical technique that allows a multi-colour visualisation of target cells in a tissue to either differentiate or highlight certain aspects within. Double-staining is helpful when running IHC panels for the diagnosis of various conditions, including the differing skin cancer subtypes.

Aim

To develop an in-house diagnostic IHC panel to differentiate skin cancers through the use of single and double staining methods.

Method

The research spans 12 weeks of laboratory work conducted at RMIT University between a group of 4 students. Archived formalin fixed paraffin embedded (FFPE) patient tissue samples donated to RMIT University were used for the differentiation of BCC, SCC and Melanoma human skin cancers. The antibodies used for immunohistochemistry (IHC) included EMA, CEA, BerEP4, BCL2, HMB45 and s100, which were analysed for expression and compared.

Results

The double-label immunohistochemical panel was successful in aiding the diagnosis of the patient samples. S100 and HMB45 were run in conjunction with one another forming the double-stain for the diagnosis of melanoma. CEA and EMA were also run as a double-stain allowing for accurate diagnosis of SCC. BerEP4 displayed strong positive staining for BCC and negative staining for SCC.

Conclusion

After routine staining of patient samples, a panel of single and double staining IHC antibodies were formulated to be able to differentiate BCC, SCC and melanoma. Alongside H&E staining which accurately demonstrates tissue morphology, IHC has also shown to provide useful information that can assist with diagnosis.

P-38 MODELLING MOTOR NEURON FUNCTIONALITY USING NEURAL CELL LINES

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Introduction
Cell lines are commonly used for in vitro studies to model cellular responses to stimulants and their involvement in specific neural functionalities.

Aims
Three cell lines (NG108-15, NSC34 and PC12s) were investigated to identify the most applicable to model motor neuron formation and function in neuromuscular junctions (NMJs).

Methods
Multielectrode array studies and calcium imaging were used to determine the electrical functionality of the cell lines at various time points of the neuro-differentiation. Protein expression of neurotransmitter markers were assessed to identify simulatability of the cell lines and their ability to express acetylcholine.

Results
Calcium flux across the plasma membrane was prevalent from the first week of culture. The protein markers were likewise expressed in the differentiated cells from the first week and increased in intensity in a temporal manner. PC12s have shown minimal neurite outgrowth relative to NSC34 and NG108 cell lines.

Conclusion
The cell lines exhibit characteristics of motor neurons however further work is needed to control their functionality.

P-39 METABOTROPIC GLUTAMATE RECEPTOR 1 IS INVOLVED IN NEURAL CONTROL OF COLONIC MOTILITY

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Introduction
Group I metabotropic glutamate receptors (mGluRs) comprise mGluR1 and mGluR5 and have well-established roles in excitatory neurotransmission and synaptic plasticity in the central nervous system (CNS). Previous research has shown Group I mGluRs have similar roles in excitatory neurotransmission in the network of neurons located in the gut wall, known as the enteric nervous system (ENS), which controls gastrointestinal motility. However, the individual roles of mGluR1 and mGluR5 in gastrointestinal function remain unknown.

Aims
We aimed to elucidate the effect of blocking mGluR1 and mGluR5 on neurally-mediated propagating contractions of the gut, known as colonic migrating motor complexes (CMMCs).

Methods
As the ENS can function autonomously in the absence of CNS input, CMMCs were examined in the isolated colon. Specifically, the colons of male and female mice aged between 7-12 weeks were dissected and placed in a heated organ bath containing Krebs solution. A video camera recorded four 15-minute videos of CMMCs in the presence of the Group I mGluR antagonist PHCCC (30 μM), as well as specific antagonists against mGluR1 (BAY 36-7620, 10 μM) and mGluR5 (MPEP, 10 μM). These videos were then converted into spatiotemporal maps and analysed for CMMC frequency, length and speed.

Results
Exposure to PHCCC led to a significant decrease in the frequency of CMMCs (Control: 3.8 ± 0.5 CMMCs/15 min; PHCCC: 2.1 ± 0.4 CMMCs/15 min, n=11, p=0.004), CMMC length (Control: 37.0 ± 2.1 mm, n=9; PHCCC: 22.0 ± 4.8 mm, n=7, p=0.01), and CMMC speed (Control: 2.1 ± 0.6 mm/s; PHCCC: 0.9 ± 0.3 mm/s, n=7, p=0.02). Similarly, when the colon was exposed to BAY 36-7620, we also found decreased CMMC frequency (Control: 8.8 ± 0.9 CMMCs/15 min; BAY 36-7620: 4.1 ± 1.0 CMMCs/15 min, n=8, p=0.03), CMMC length (Control: 42.4 ± 1.3 mm; BAY 36-7620: 33.9 ± 2.7 mm, n=8, p=0.03), and decreased CMMC speed (Control: 1.9 ± 0.2 mm/s; BAY 36-7620: 1.3 ± 0.2 mm/s, n=8, p=0.03). However in the presence of MPEP, there was no significant change in CMMC frequency (Control: 4.2 ± 0.6 CMMCs/15 min; MPEP: 2.6 ± 0.4 CMMCs/15 min, n=5, p=0.06), length (Control: 38.6 ± 2.7 mm; MPEP: 34.03 ± 4.3 mm, n=5, p=0.07), or speed (Control: 1.4 ± 0.1 mm/s; MPEP: 1.1 ± 0.1 mm/s, n=5, p=0.13).

Conclusion
These data suggest mGluR1, but not mGluR5, is involved in both the initiation of CMMCs and propagation of colonic contractions.

P-40 EVALUATION OF LONG READ DIRECT RNA SEQUENCING WITH NANOPORE TECHNOLOGY TO DETECT AND QUANTIFY GENE ISOFORMS

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Introduction
Studying the expression of gene isoforms remains challenging as current sequencing technology relies on the fragmentation of nucleic acids into short reads. In contrast, Oxford Nanopore Technology (ONT) recently demonstrated their technology can be used to directly sequence RNA (dRNA-Seq), without the fragmentation steps, PCR amplification or conversion to cDNA required in standard sequencing methods such as Illumina. dRNA-Seq has the potential to revolutionise the field of biology, allowing sequencing of full-length transcripts to study alternative splicing patterns and analyse how different gene isoforms contribute to diseases such as schizophrenia.

Aims
Assess the ability of ONT dRNA-Seq to detect differential gene expression between cell types and identify alternatively spliced gene isoforms. Build a bioinformatic framework for future studies characterising known risk genes in neuropsychiatric disorders.

Methods
This study utilised the well-established SH-SH5Y cell culture model of neuronal differentiation. RNA was extracted from differentiated and undifferentiated SH-SY5Y cells, synthetic spike-in RNA was added to each sample and these were sequenced with ONT. Reads were mapped to the human genome (hg38) and synthetic genome using *minimap2*. Genes and transcripts were then quantified using *featureCounts* and *Salmon* respectively. Differential expression (DE) analysis was performed to determine if direct RNA sequencing sensitivity was sufficient to detect DE at the gene and transcript level and quantify known synthetic alternative isoforms.

Results
The median read length was 1,013 nucleotides, and the longest read found was 16,019 nucleotides. Differential expression between differentiated and undifferentiated cell populations was detected, and distinct sample groups clustered separately at the gene and transcript level. Genes significantly (adj-$p<0.01$) upregulated in differentiated samples were used in a gene ontology (GO) analysis. Genes implicated in axon guidance were highly associated with differentiated SH-SY5Y cells, validating the ability of the sequencing method to identify biologically relevant changes in gene expression. Synthetic alternative isoforms were detected and the ability to quantify these was tested with a linear regression of the known $\log_2$ fold change between samples against the observed fold change. The R^2 was 0.88 at the gene level and 0.72 at transcript level.

Conclusions
dRNA-Seq with ONT can reliably detect alternatively spliced gene isoforms and quantify these accurately against a known spike-in control. The technology can be applied to better characterise gene isoforms involved in disease, particularly in the brain which has a complex splicing program. These new methods make it possible to analyse transcript isoforms without reconstructing or inferring them from short read data.

P-41 INTERPRETABLE MACHINE LEARNING METHODS FOR THE CHARACTERISATION AND CLASSIFICATION OF SKIN CANCER

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Introduction
Deep Learning algorithms have been shown to outperform expert humans in classification problems, but remain highly criticised for being "black boxes". To integrate this technology successfully, we need machine decision processes to not just correlate with human decisions, but be implemented so that they can be interrogated and explained.

Aims and Methods
Our approach is to train deep neural networks to perform dense classification tasks, assigning each pixel in an image to a meaningful class. By constraining the entire input domain, the network is forced to learn representations that are explicable to humans. These interpretable representations can then be used to perform an overall classification, as well as routine characterisation tasks such as measuring surgical margin clearance. To test our hypothesis, we used 290 histological images of non-melanoma skin cancer: Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC), and Intraepidermal Carcinoma (IEC). For each image, we hand-annotated pixels into 12 classes: Glands, Inflammation, Follicles, Hypodermis, Reticular, Papillary Dermis, Hypodermis, Keratin, Background, BCC, SCC, and IEC.

Results
Our best network achieved an overall pixel accuracy of 85.3%, producing high quality and interpretable segmentations. Using these outputs to perform a per-image classification (Healthy, BCC, SCC, IEC), we achieved an accuracy of 96.6% (no false-negatives) and 99.1% for Cancer versus Non-Cancer. We also demonstrate the ability to automatically assess surgical margin clearance which can dramatically improve pathologist efficiency.

Conclusion
Our novel results provide the interpretability that is necessary for healthcare and showcase for the first time how these systems can be meaningfully integrated into the dermatopathologist workflow.

P-42 AUTOMATIC DETECTION OF TASK-BASED VISUAL FEATURES IN PROSTHETIC VISION

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Introduction
People with retinitis pigmentosa and age-related macular degeneration gradually lose their vision due to atrophy of photoreceptors - the cells in the retina that convert light energy into electrochemical neural signals. It is possible to restore rudimentary vision to these people by electrically stimulating remaining neurons in the retina via an implanted electrode array. There are several limitations of current visual prostheses, including the low number of stimulating electrodes and poor resolution of perceived images.

Aims
One way of improving a functional outcome to a user is to enhance the perceived image by selecting a limited number of important features in the image (such as edges). These features are then converted into a stimulation strategy and the retina is stimulated accordingly. The features are usually hand-crafted (e.g., contrast enhancement, depth-based etc) and while visually salient, do not necessarily correspond to the highest priority needs of the task being performed by a visual implant user. In this work, we propose a method for identifying salient task-based visual features.

Methods
We train a virtual agent using deep reinforcement learning to learn a representation of its environment that facilitates its task, i.e. navigation. From the convolutional layers in the deep neural network that maps images to action choices, we extract feature maps that encode a visual representation of the environment. These feature maps are then used to filter real-world images to highlight task-relevant features in the captured images.

Results
Our results show that the learnt representations are indeed salient to the agent's action choices, and crucially, can be transferred as filters to be applied to real images that resemble the structure of the simulated environment. For navigation, sailent features include major structural edges, colour contrasting and plane detection. This algorithm ensures that implant recipients are visualising elements of their environment that directly facilitate their ability to perform certain tasks.

Conclusions
The proposed algorithm offers the opportunity to learn low-dimensional encodings of vision-to-action mappings for a given task. The algorithm is inherently task-based and allows automatic feature selection, and is scalable to any basic vision-guided capability. This may enhance functional outcomes for a visual implant user.

P-43 IMPROVING CANCER IMMUNOTHERAPIES BY UNDERSTANDING THE CROSSTALK BETWEEN HUMAN NEUTROPHILS AND CD8⁺ T CELLS

★ ***ExoPharm travel award***

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Introduction

Neutrophils are the most abundant type of leukocytes and traditionally considered the first line in host defence. In previous studies, it was demonstrated that subsets of neutrophils can have either anti-tumoural or pro-tumoural properties in murine models of cancer. This dual role of neutrophils evokes questions about their ability to participate in the adaptive immune system beyond their established role in the innate immune system. We have previously shown, that neutrophils acquire an immune suppressive phenotype in T cell inflamed environments and thus limit the efficacy of cancer immunotherapies. Whether these findings can be translated to human neutrophils remains unknown.

Aims

The main aim of this study is to determine if human neutrophils also acquire immunosuppressive properties upon exposure to activated CD8⁺ T cells.
Our second aim is to understand the cell interactions between neutrophils and CD8⁺ T cells.
Finally, we will analyze the neutrophil immunosuppressive phenotype from a transcriptomic perspective.

Methods

To test our hypothesis experimentally, neutrophils were isolated from whole blood of healthy donors using the MACSxpress ® Neutrophil Isolation Kit and MACSxpress ® Erythrocyte Depletion Kit. Subsequently, neutrophils were cultured in control cRPMI media or in T-cell conditioned media (TCM) for 24 h, 48 h or 72 h. After each time point, the phenotype of neutrophils was analyzed using fluorescent associated-cell sorting (FACS).
To understand the cell interactions between both populations, we proposed a proliferation assay with Cell Trace Violet (CTV) for 96h. In this assay, it was compared to the proliferation of CD8⁺ T cells cultivated alone respective from a co-culture with neutrophils.
RNA sequencing was performed from the culture of 24h of neutrophils with control media and with the TCM media.

Results

In line with our previous findings in murine neutrophils, human neutrophils also showed significant upregulation of molecules associated with immune suppression including PD-L1, Galcetin-9 and CD39 after incubation with TCM compared to control medium. More importantly, human neutrophils also showed increased expression of the receptor tyrosine kinase c-MET, which we have shown is a promising target to inhibit neutrophil migration.
From the proliferation assay it was showed a clear suppression of CD8⁺ T cells when they were in co-culture with neutrophils.

Conclusion

To conclude, activated CD8⁺ T-cells secrete signalling cues that influence the phenotype of healthy human neutrophils in vitro. Future experiments implying the co-culture of CD8⁺ T-cells and neutrophils will shed more light on this relationship and how existing immunotherapies can be improved by targeting neutrophils.

P-44 DEVELOPMENT OF A DOUBLE-LABEL IMMUNOHISTOCHEMISTRY PANEL FOR THE DIAGNOSIS OF MELANOMA AND NON-MELANOMA SKIN CANCERS

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Introduction

Melanoma is one of the most aggressive types of skin cancer, whose histological features can sometimes mimic other tumours. Melanoma begins in the melanocytes located in the bottom layer of the epidermis and often spreads to other parts of the body. Beside Melanoma, Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC) are two common forms of non-melanoma skin cancers that each present with different histological features. Immunohistochemistry (IHC), especially Double-label IHC staining, has been a very effective diagnostic tool in identifying masses, including both melanoma and non-melanoma skin cancers. It provides doctors and patients with an accurate diagnosis that can lead to more appropriate treatment options. Therefore, developing a double-label IHC panel can significantly assist with the diagnostic process and provide clinicians with a more accurate and reliable analysis of varying tumours.

Aim

This project aims to produce a double-label IHC panel that identifies melanoma and non-melanoma skin cancer tumours. Furthermore, it aims to discover the optimal working conditions (i.e. pH, temperature, antigen retrieval method) for the target double-label IHC markers in order to obtain the best staining quality for all antibodies used.

Methods

The project utilises various histological methods including embedding, microtomy, routine H&E, and IHC (DAKO) procedures. The target double-label IHC stains are chosen and tested from a panel of melanoma and non-melanoma skin cancer markers including S100, HMB45, CEA, EMA, BerEP4, BCL-2, and CD10. Both negative and positive controls are used throughout this project to ensure the accuracy and reliability of results achieved. Samples used in the projects are human skin tissues donated to RMIT by partner hospital laboratories. Antibodies used in the project are DAKO's ready to use (RTU) antibodies.

Results

The project has successfully produced a double-label IHC panel for the diagnosis of melanoma and non-melanoma skin cancers.

Conclusion

In summary, this project has established combinations of skin cancer markers using IHC that are able to identify melanoma and non-melanoma skin cancers. Additionally, it has proven that IHC is a powerful and effective tool in detecting various skin cancers and distinguishing between various key characteristics present in each. It provides pathologists with reliable results to provide the most accurate diagnosis, prognosis and suitable treatments for patients.

P-45 CLEC9A MEDIATED ANTIGEN PRESENTATION BY CONVENTIONAL DC1 FACILITATES B CELL ACTIVATION AND ANTIGEN PRESENTATION FOR ENHANCED HUMORAL IMMUNITY

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Introduction
Induction of potent humoral responses is a feature of most successful vaccines. It is known that various dendritic cell (DC) subsets support humoral immunity by inducing $CD4^+$ T helper cells (T_{FH}), but their role in supporting B cell activation is less well defined. The fact that antigen (Ag) targeted to Clec9A on $CD8^+$ conventional DCs (cDC1) induces strong humoral responses even in the absence of adjuvant, implicates cDC1 in humoral responses.

Aims
We hypothesised that Ag delivered to Clec9A not only benefits T cell priming but also facilitates activation of B cells.

Methods
To further explore this, we made use of transgenic B and T cell populations and engineered anti-Clec9A antibodies to contain either T cell epitopes only, or both T and B cell epitopes, to target antigen to T cells alone or concomitantly to B and T cells.

Results
We observed that Ag targeted to Clec9A was retained in its native form on the surface of cDC1, which allowed direct delivery of Ag to B cells and induction of B cell activation. This enabled B cell migration to the T/B border, which was essential to support T_{FH} development**.** Interestingly, we found that Clec9A mediated Ag-presentation by cDC1 did not enable increased Ag uptake by B cells relative to the uptake of soluble untargeted Ag, but it did enhance B cell activation and subsequent Ag degradation and presentation. Our findings suggest that soluble Ag is poorly processed by B cells even when the same B cell is co-activated by a Clec9A-targeted antigen.

Conclusion
These findings suggest cDC1 are capable of generating humoral responses and that the role of individual DC subsets may be dictated by the ability to display the tested antigen. Targeting Ag to Clec9A on cDC1 represents an efficient mechanism for B cell activation and generation of humoral immunity, which can be exploited by novel vaccination approaches.

P-46 DIFFERENTIAL REGULATION OF CCL17 BY GM-CSF AND IL4

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Introduction

Rheumatoid Arthritis (RA) is a complex autoimmune disease affecting up to 1% of the total global population. Its characteristic chronic inflammation is thought to be perpetuated by the imbalance of helper 17 T cells (Th17) and regulatory T cells (Tregs) in the synovial fluid (SF) of RA patients. These cells also express CC chemokine receptor 4 (CCR4), which has C-C motif ligand 17 (CCL17) as one of its known ligands.

CCL17 is required for granulocyte-macrophage-colony-stimulating-factor (GM-CSF) driven inflammatory arthritic pain, and both are found at elevated levels in the SF of RA patients. GM-CSF and interleukin-4 (IL4) upregulate CCL17 levels in human monocytes and mouse macrophages via interferon regulatory factor 4 (IRF4), despite being pro- and anti-inflammatory cytokines, respectively. Interestingly, these cytokines also differentiate monocytes into dendritic cells (mDCs), with CD1c+mDC differentiation also dependent on IRF4. Notably, the CD1c+mDCs population is elevated in RA patients, correlating with increased CCL17 production.

Aims

In this study, the role of GM-CSF and IL4 on CD1c+DC differentiation and CCL17 regulation was investigated.

Methods

Human monocytes and murine macrophages were isolated and treated with either GM-CSF or IL4 alone or together for up to 16 hours. Western Blot and ELISA were used to analyse protein concentrations. Quantitative PCR was used to measure gene expression. Flow cytometry was used to measure the extracellular receptors CD1c and CD14 on human cells.

Results

We found that IL4 had no significant effect on the GM-CSF-promoted CD1c+mDC numbers; however, despite being able to upregulate CCL17 alone, the addition of IL4 to GM-CSF-treated monocytes significantly suppressed CCL17 levels. These findings indicate that the elevated levels of CCL17 in the SF of RA patients could be due to the differentiation of CD1c+mDCs in an inflammatory environment dominated by GM-CSF. By targeting GM-CSF, CCL17 or CD1c+mDC, Th17 cell numbers could be reduced and homeostasis could be restored in affected joints.

P-47 ANALYSIS OF IMMUNE MARKERS IN HUMAN SKIN CANCERS

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Introduction

The immune system plays an important role in maintaining the tumour microenvironment, which may suppress or enhance the growth and metastasis of tumour cells. Skin cancers are divided into two main groups, melanoma and non-melanoma (including squamous cells and basal cell carcinoma). Immune cells contribute to the tumour microenvironment of skin cancer, which tend to grow faster and more aggressive in patients with the weaker immune system, and are more likely to be fatal.

The tumour microenvironment contains different kinds of immune cells, including innate immune cells and adaptive immune cells. The most frequently found immune cells in tumour microenvironment are tumour-associated macrophages (TAMs) and T cells. Stroma consists of different components that surround the cancer cells allowed direct contact to the cytokine and chemokine production, which could control the growth and shape of tumour. A suppressed immune system can diminish the activity of natural killer T cells (NKT), limiting resistance towards tumour growth. Cancerous bodies can also directly disrupt these T cells by using inhibitory molecules, suppressing a specific cell without affecting the whole immune system. PDL1 is an antigen that can suppress the function of cytotoxic killer T cells.

Aims

The aim of this project was to analyse the frequency and intensity of immune markers in human skin cancers, including basal and squamous cell carcinoma as well as melanoma.

Methods

Archival human skin tumour samples were provided by the RMIT University Pathology Unit and various antibodies against various immune cell markers were investigated using immunohistochemistry (IHC) staining method. Antibodies against CD markers, cytokeratin markers and PDL1 were analysed. Human tissue was cut, stained by H&E method in order to select appropriate tissue samples and stained using IHC. Staining for each antibody was analysed for expression and intensity and presented.

Results

The study shows expression of various immune cell markers may have diagnostic significant as well as an opportunity to identify new targets for future therapy.

P-48 DCLK1: A NOVEL PROMOTER OF GASTRIC CANCER PROGRESSION

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Introduction
Doublecortin-like kinase1 (DCLK1), a microtubule-associated protein (MAP), recently gained interest in the cancer research field. Whole-genome sequencing suggests that DCLK1 is a novel tumour driver and DCLK1 overexpression correlates with epithelial-to-mesenchymal transition (EMT) in pancreatic and colon cancer. A recent meta-analysis of 2660 patients from 12 different tumour types, showed that *DCLK1* overexpression correlates with advanced and poorly differentiated cancers, lymph node metastasis and reduced overall patient survival.

Aims
Our aim is to investigate how DCLK1 in GC contributes to cancer progression and whether DCLK1 could be a potential novel target for GCs.

Methods
The Cancer Genome Atlas (TCGA) stomach adenocarcinoma (STAD) RNAseq dataset was analysed using R-studio to generate unsupervised clustered heatmaps and spearman correlations to visualize *DCLK1*-expression in combination with EMT, ECM, Metastasis, angiogenesis, and immune-cell markers. For clinical relevance, patients were mapped to their molecular subtype, histological classification and clinical staging. Currently, we are evaluating DCLK1 expression of 300 gastric cancer patients by immunohistochemistry (IHC) using tissue microarrays (TMAs). We established a DCLK1-overexpressing MKN1 gastric cancer cell line to investigate the cellular effects of high DCLK1 levels using *in vitro* and *in vivo* proliferation, migration and invasion assays, and whether the effects are reversible using a DCLK1-inhibitor.

Results
Our analysis of the TCGA-STAD dataset showed that *DCLK1*-high expressing tumours significantly clustered within the genomic stable molecular subtype and the histologically diffuse type. The DCLK1-overexpression increased migration and invasion *in vitro* and *in vivo*. These findings support our TCGA-STAD data analysis where high *DCLK1* correlates with increased expression of EMT markers. Strikingly, we observed an overall increase in chemokine secretion *in vivo* after DCLK1-overexpression with CXCL12 as the main upregulated chemokine. This is supported by findings in the TCGA-STAD data set, where *DCLK1* levels significantly correlate with *CXCL12* levels. Furthermore, a DCLK1-inhibitor reversed migration, invasion and chemokine secretion in the DCLK1-overexpressing MKN1 cells to parental MKN1 cell levels. *Alt*ogether, our results suggest that DCLK1 may be a good target for poor prognosis GCs with high DCLK1 levels.

Conclusion
In conclusion, DCLK1 overexpression correlates with poor patient survival, and increased invasion, migration, EMT and chemokine secretion. Thus far, the signalling cascade in which DCLK1 can induce an EMT or increase chemokine secretion is poorly understood. Our aim is to address these questions using SILAC mass spectrometry studies by comparing total cell proteome, phospho-proteome and secretome datasets of parental MKN1 and DCLK1-overexpressing MKN1 cells, treated with the DCLK1-inhibitor and untreated.

P-49 HISTONE DEACETYLASE INHIBITORS (HDACi) AS A DIFFERENTIATION THERAPY FOR NON SMALL CELL LUNG CANCER

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Introduction

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. 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 tissue of the body, collectively occurring in >25% of all human malignancies. Non small cell lung cancer (NSCLC) accounts for 40% of all lung cancers and has an overall 5-year survival. Mutations in SMARCA4 are believed to occur in 6% of NSCLC.

Results

Using a panel of SMARCA4-wildtype and mutant human NSCLC cell lines, we show a marked sensitivity for the HDACi, panobinostat, in SMARCA4-null and SMARCA4-knockout NSCLC cell lines. Furthermore, sustained low-dose panobinostat treatment of the SMARCA4-null NSCLC cell line, A549, results in gene expression changes consistent with differentiation, including the downregulation 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, as compared to SMARCA4-wildtype NSCLC H441 xenograft model. Furthermore, similar xenograft model with H441 with SMARCA4 knock-out is sensitive to panobinostat treatment and demonstrated similar phenotype with A549 xenograft models, in which mice have reduced tumour growth rate and longer survival rate, as compared to its parental cell line xenograft model. Together, these data suggest that low-dose panobinostat promotes differentiation of SMARCA4-null NSCLC.

P-50 ANALYSIS OF EPITHELIAL - MESENCHYMAL TRANSITION (EMT) MARKERS IN HUMAN SKIN CANCERS

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Introduction

In Australia, skin cancer has the highest incidence in the world. Moreover, the cost related to skin cancer is the highest of all cancers. The three most common types of skin cancer are Melanoma, Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC). Therefore, skin cancer can be categorised into two types; melanoma which starts from melanocytes and non-melanoma skin cancer (NMCS) which starts from skin cells such as squamous cell, basal cell and/or Merkel cells. The reason for this classification is that melanoma is known to be more life-threatening than NMCS and the treatment approaches are also different. Thus, in order to get more cost-effective skin cancer management, early diagnosis and differentiation of skin cancer are important.

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their apical-basal polarity and cell adhesion. Epithelial cells also gain the ability for migration and invasion resulting in the rearrangement of the cytoskeleton. It has been proven that EMT is highly associated with cancer metastases. Therefore, EMT markers play an important role in the differentiation of different skin cancers. It involves the down-regulation of epithelial marker such as E-cadherin (E-CAD) marker. It also increases the expression of other mesenchymal markers (eg: Vimentin (VIM), SNAI1 and Fibronectin).

Aim

To study the immunohistochemistry (IHC) expression of different EMT-related biomarkers in different types and stages of human skin cancer. This is in order to analyse the potential role of EMT-related biomarkers in the early stage of skin cancer differentiation and diagnosis.

Method

The EMT markers investigated were E-CAD, EMA, SOX10, A-SMA, and VIM on archival human skin cancer blocks including SCC, BCC and Melanoma. Control tissues inclusive of positive and negative controls used alongside each marker as quality control. The use of IHC techniques and slide image analysis to analyse the representation, frequency and intensity of each marker in the various types of skin cancers to determine the specificity and up/down-regulation.

Results

In the experimental process of investigating the presentation of EMT biomarkers in the various types of skin cancers, there were variations that were analysed. Frequency and intensity variations of these EMT related biomarkers were presented and compared.

Conclusion

EMT markers can be a useful tool to differentiate between different human skin cancer types (squamous cell carcinoma, basal cell carcinoma and melanoma).

P-51 ADVANCED GLYCATION END-PRODUCTS& THE PREIMPLANTATION EMBRO: EFFECTS OF DIETARY FACTORS ASSOCIATED WITH OBESITY

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Introduction
Obesity is a global pandemic: 63% of Australians are overweight or obese. Critically, approximately 30% of Australian women are obese before conception (AIHW). Obesity reduces natural and IVF pregnancy success, and increases miscarriage & preeclampsia risk. 'Toxic' Advanced Glycation End-Products (AGEs), formed when reducing sugars react with the free amino group on proteins, are elevated four-fold in uterine fluid of obese, infertile women versus lean women.

Aim
To examine if AGEs, equimolar to those within the uterus of obese women, impact the development and function of preimplantation embryos.

Method
Preimplantation mouse embryos were cultured with AGEs equimolar with uterine fluid concentrations from lean versus obese women. Developmental morphokinetics were assessed by time-lapse microscopy (Embryoscope). Trophectoderm (TE) and inner cell mass (ICM) cellular allocation was determined by differential staining. AGEs receptor (RAGE & TLR4) immunolocalisation, & TUNEL assay (apoptotic index assessment), investigated the potential mechanism of AGEs action on embryo development. Implantation potential was assessed by blastocyst outgrowth assay. To ameliorate the effects of AGEs, embryos were co-cultured with the RAGE antagonist FPS-ZM1.

Results
Preimplantation embryos express RAGE & TLR4, providing a mechanism for AGEs-mediated signalling. "Obese" AGEs significantly reduced blastocyst hatching rates (50%, $p<0.0001$) and embryonic cell-proliferation, supported by the absence of TUNEL-staining and delay in morphokinetic embryo-development (~3-hour lag). AGEs reduced blastocyst cell number (24%, $p<0.0001$), specifically in the trophectoderm (23%, $p<0.001$). Blastocyst outgrowth, an indicator of implantation potential, is compromised by "obese" AGEs (~30%, $p<0.01$). RAGE antagonism reduces AGEs mediated effects on embryo development by preventing the reduction in trophectoderm cell numbers.

Conclusion
Elevated uterine AGEs are likely detrimental to fertility. AGEs equimolar to the obese uterine environment detrimentally affect early mouse embryo development, blastocyst hatching, and implantation potential, providing a physiological link between obesity and reduced fertility. RAGE antagonism partially inhibited these effects, providing a potential therapeutic option to improve embryo development in obese infertile women.

P-52 NON-SPECIFIC IgA INHIBITS HIV BROADLY NEUTRALIZING ANTIBODY (bnAb) FC FUNCTIONS

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Introduction
Human immunoglobulin A (IgA) is an antibody found in mucosal secretions and plasma as two isotypes; IgA1 and IgA2. Literature suggests an important role of mucosal IgA in protection from HIV-1 infection in macaque SHIV models and some human cohorts. However, the role of plasma IgA in HIV-1 protection and disease progression is controversial. This was highlighted in the moderately protective human HIV-1 RV144 vaccine trial (31.2%) that associated immunoglobulin G (IgG) Fc functions, including antibody-dependent cellular cytotoxicity (ADCC), with protection from HIV-1 infection. Interestingly, RV144 induced plasma IgA to HIV envelope (gp120), reduced Fc capacity (ADCC) and reduced vaccine efficacy via epitope competition with protective HIV-specific IgG. Passive transfer of certain broadly neutralizing antibodies (BnAbs) in macaques have shown that both neutralizing and Fc functions are required for protection. However, the effect of plasma IgA on the Fc function of BnAbs is yet to be studied.

Aims
Here we endeavour to determine if plasma IgA influences the Fc functions, specifically the phagocytic capacity, of various BnAbs and HIV positive IgG.

Methods
Pooled HIV positive IgA (n=10) and pooled HIV negative total IgA (n=6), IgA1, IgA2 and colostrum IgA were used to assess the inhibitory role of IgA on the ability of HIV-specific BnAbs and HIV positive IgG to induce gp120-specific antibody-dependent cellular phagocytosis (ADCP) using high throughput flow cytometry.

Results
The addition of HIV negative total IgA (median=7.05%, IQR=5.00%, p=0.0500), IgA1 (median=16.91%, IQR=7.63%, p=0.0103), IgA2 (median=16.22%, IQR=2.96%, p=0.0006) and colostrum IgA (median=22.41%, IQR=8.61%, p=0.0004) significantly reduced ADCP compared to responses of various BnAbs and HIV positive IgG alone. The addition of HIV positive IgA (median=6.32%, IQR=6.49%, p=0.0724) did not significantly reduce ADCP compared to responses of various BnAbs and HIV positive IgG alone.

Conclusions
Preliminary findings indicate IgA is capable of reducing IgG-mediated ADCP *in vitro*. HIV negative IgA, derived from plasma and colostrum, cannot bind specifically to gp120. Therefore, this inhibitory mechanism is not mediated by IgA epitope competition with IgG. Instead, IgA may inhibit BnAb Fc functions in a nonspecific manner, potentially via Fc alpha receptor binding (FcαR) through inhibitory cellular pathways. Future work aims to investigate this mechanism. This data indicates that non-specific IgA can influence BnAb Fc functions and should not be overlooked in passive transfer studies.

P-53 DNA METHYLTRANSFERASE 3A (DNMT3A) PROMOTES INFLAMMATION-ASSOCIATED GASTRIC CANCER GROWTH

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Introduction
Gastric cancer (GC) remains the third leading cause of cancer-related death worldwide. While inflammation is a well-established driver of gastric tumorigenesis, only a small subset of GC patients respond to immune checkpoint therapy. The understanding of the pro-inflammatory tumour microenvironment, which suppresses anti-tumour immune responses, is a field of great interest. One proposed mechanism of immune escape is silencing tumour-antigen expression and thereby avoiding immune recognition. DNA methyltransferases (DNMTs) are the enzymes responsible for epigenetic silencing of gene expression and are overexpressed in various tumours.

Aim
Here we are studying the role of DNMT3A in gastric cancer mouse models and its potential as a therapeutic target.

Methods
We conducted a stomach-specific *Sleeping Beauty* (SB) transposon mutagenesis screen in inflammation-sensitized mice ($gp130^{F/+}$). To study the functional and mechanistic contribution of an identified cancer-driver gene (*Dnmt3a*) to GC growth, we established a *Dnmt3a*-overexpressing inflammation-driven gastric cancer mouse model ($gp130^{FF}$, $A33^{Dnmt3a}$). In addition, we investigated expression levels in a second gastric cancer mouse model (*KPT*), where mutant *Kras, Pi3kca* and *Tp53* expression results in highly advanced invasive GC formation.

Results
We identified *Dnmt3a* as a top ten cancer-driver gene in our SB mutagenesis screen, where transposon-mediated mutation of *Dnmt3a* led to spontaneous gastric adenoma formation. We and others detected DNMT3A overexpression in human gastric cancer specimen. To study the functional and mechanistic contribution of DNMT3A to gastric tumour growth, we established a *Dnmt3a*-overexpressing inflammation-driven gastric cancer mouse model ($gp130^{FF}$, $A33^{Dnmt3a}$), where $gp130^{FF}$ mutant gastric tumours have a 10-fold elevated *Dnmt3a* expression. Importantly, tumour-specific *Dnmt3a* overexpression significantly increased gastric tumour burden. We are currently investigating the effects of *Dnmt3a* overexpression on the tumour-associated immune cell composition in this model. In a second mouse model, where mutant *Kras, Pi3kca* and *Tp53* expression results in highly advanced invasive gastric carcinoma formation, we have identified DNMT3A as being highly expressed in the invasive front of tumours as well as in their liver metastases. We are currently establishing DNMT3A-deficient (*Kras-, Pi3kca-, Tp53*-mutant) tumour organoid models to study the effects of DNA methylation on tumour progression, immune escape and therapy responses.

Conclusion
Taken together, we provide evidence for a GC-driver function of *Dnmt3a*, which encourages further studies to investigate the potential of DNMT3A as a novel target for monotherapy or in combination with immunotherapy in GC.

P-54 INVESTIGATING THE ROLE OF TIMELESS IN BREAST CANCER

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Introduction

Breast cancer is one of the most common causes of cancer death in females, accounting for 25% of new cancers in Australian women. Most patients have an oestrogen-dependent disease that responds to selective estrogen receptor (ER) modulators (SERMs) such as tamoxifen. Tamoxifen is a mainstay of adjuvant treatment, however many patients either do not respond or eventually become resistant to the drug. There is currently no way to predict the outcome of tamoxifen. Previous profiling work identified timeless as a potential predictor of tamoxifen response, but the mechanisms underlying this association are unknown. We recently showed that timeless can bind to ER and increase its activity, thereby implicating this protein in oestrogen/tamoxifen signalling.

Aim

In order to understand the role of timeless in oestrogen signalling in breast cancer, our first aim was to quantify timeless expression in a panel of ER+ and ER- breast cancer cell lines. We hypothesized that timeless expression would be higher in ER+ cells since timeless is known to be induced by oestradiol. Our second aim was to generate stable cell lines that over-express timeless, for use in functional assays.

Method

We quantified timeless mRNA expression in a panel of breast cancer cell lines (T47D, MCF7, ZR-75-1, MDA-MB468) by real-time PCR, using the ddCT method.
Stable MCF7, T47D and MDA-MB468 cells overexpressing timeless were generated after transfecting cells with pcDNA4Flag-Timeless (or the parent pcDNA4 vector) and selection with Zeocin. Cells were then maintained in culture with 100 ng/ml Zeocin. Validation of Tim-overexpressed cells in RNA level was performed by quantitative PCR/ real-time PCR.

Results

Timeless was expressed in all cell lines, both ER+ (T47D, MCF7, ZR-75-1) and ER- (MDA-MB468). No significant difference in expression across the cell lines was observed. After transfecting timeless into cell lines, timeless expression was increased by 3-fold, 2-fold, and 12-fold in T47D, MCF7, and MDAMB486 cells, respectively.

Conclusion

Timeless was expressed at a similar level in all cell lines studies, independent of ER status. Therefore, although timeless is a known ER target gene, it is not dependent on ER for basal expression. Further, we successfully overexpressed timeless in each cell line. These cells will aid future work to identify the effects of timeless on oestrogen signalling, cell proliferation and breast cancer cell function.

P-55 DNA CONSERVATION IN BONE MARROW TREPHINE

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Introduction
Molecular testing is becoming increasingly important in pathology, for both diagnosis and treatment options. Current decalcification procedures degrade the deoxyribonucleic acid (DNA) within bone marrow trephine biopsies (BMT), compromising the viability of the biopsies for molecular testing. Bone marrow aspirates (BMA) are typically used for molecular tests, but occasionally the aspirate yields a dry tap, and peripheral blood must be used for these investigations. Peripheral blood is not ideal, as the cell-free DNA contains more than just the DNA from the targeted tumour tissue but also DNA from normal cells, resulting in low specificity.

Aims
This study investigates alternative BMT decalcification methods through the use of ethylenediaminetetraacetic acid (EDTA) based decalcification solution in addition to the manipulation of factors including temperature and agitation, with the goal of enabling molecular tests to be conducted on formalin-fixed paraffin embedded (FFPE) BMT tissue.

Methods
Formalin fixed BMT were treated with Osteosoft EDTA decalcification solution, and heated at 27^{o}C, 37^{o}C, and 50^{o}C, in conjunction with magnetic stirring. Samples were processed and embedded in paraffin to be tested at selected stages for DNA and RNA yield, in order to optimise the decalcification process.

Results
It was found that there was a higher yield of DNA and RNA in the formalin fixed BMT compared to the current RDO-treated process within the laboratory. Agitation and increased heat decreased the duration of decalcification required in order to obtain sufficiently decalcified BMT.

Conclusions
While the use of Osteosoft EDTA decalcification solution in the decalcification of formalin fixed BMT required a longer duration of decalcifying than the use of RDO, the DNA and RNA was conserved for molecular testing. The duration required to decalcify with the Osteosoft EDTA solution was optimised through the use of heat and agitation, reducing the time difference between both protocols and rendering the newer protocol more practicable.

P-56 GLYCOSAMINOGLYCANS IN NASAL AND ARTICULAR CARTILAGE INTERACT DIFFERENTLY WITH THEIR EXTRACELLULAR MATRICES

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Introduction

Sulphated glycosaminoglycans (sGAG) entrapped in the collagen meshwork provide compressive stiffness to the articular cartilage. Chondroitin sulphate is a predominant sGAG present in articular and nasal cartilage. It can be digested with chondroitinase ABC and the affinity of chondroitin sulphate chains to the cartilage extracellular matrix can be determined by the percentage of sGAG loss. The ability of enzymes to digest chondroitin sulphate depends on the interaction of chondroitin sulphate chains with other extracellular matrix macromolecules. Differences in these interactions according to the anatomical location of the cartilage has not been studied until now. Therefore, in this study articular and nasal cartilage were digested with chondroitinase ABC to assess such differences.

Aims

To investigate GAG-mediated extracellular interactions in articular and nasal hyaline cartilage.

Methods

Bovine articular and nasal cartilage plugs (Ø5 × 2 mm) were cored. Samples (n = 18) were divided into three groups per cartilage type: control, active, and blank. Samples were lyophilised and dry weights were measured. Active and blank groups were digested with chondroitinase ABC (0.1 U/mL at pH 8.0. for 24 hours). sGAG content of the plugs was determined with dimethyl methylene blue assay and normalised with the dry weight. Significant differences ($p<0.05$) in normalised sGAG content of the control and treated groups were compared using a one-way ANOVA test.

Results and Conclusions

Nasal cartilage shows higher sGAG content than articular cartilage (nasal: 392.61 ± 49.66, articular: 190.54 ± 36.36 µg/mg of dry weight). The articular cartilage active group lost 28% of its GAG compared to the blank group, while the nasal group lost only 2%. This indicates that chondroitinase ABC is less effective on nasal cartilage compared to articular cartilage.

Articular and nasal cartilage are hyaline cartilages, however, the activity of chondroitinase ABC on corresponding samples were markedly different. Nasal cartilage does not endure high mechanical loads as seen in joints with articular cartilage but has two times more sGAG. Nasal cartilage is reported to have ~25 - 40% of dry weight collagen content, whereas in articular cartilage it is 60 - 80%. This suggests there is less collagen to entrap sGAG in nasal cartilage. However, collagen-sGAG interactions in nasal cartilage might also be different compared to articular cartilage, resulting in reduced digestion with chondroitinase ABC. Articular cartilage has a zonally dependant structure, whereas nasal cartilage has no explicit zonal variation. The inability of chondroitinase ABC to remove sGAG from nasal cartilage could also be due to these structural differences.

P-57 ARTIFICIAL INTELLIGENCE FOR THE DETECTION OF KERATOCONUS

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Introduction
Keratoconus (KC) is a common degenerative corneal condition with onset typically in early adulthood that can lead to blindness. It represents one of the leading causes of corneal transplantation worldwide. Detection of early KC presents a challenge to the clinician as to the provision of the most appropriate treatment option. Recently, machine learning techniques have shown usefulness in ophthalmology. Application of such techniques in KC may aid in identifying features that are important in KC diagnosis and progression allowing for better targeted treatments to slow or stop its progression.

Aims
The aim of this study is to review the current and future directions of artificial intelligence (AI) and machine learning as applied to KC.

Methods
A literature search in PubMed using the keywords “keratoconus”, “machine learning”, “artificial intelligence”. Logical operators, ‘AND’ and ‘OR’ were used, and no restriction was placed on the publication year. Studies with titles or contents not directly related to the research topic were excluded, and articles that applied AI in detecting, staging or monitoring KC were selected.

Results
Thirty-five articles were identified and subsequently reviewed. The review reveals that various AI techniques using parameters derived from different corneal imaging systems have been applied for the following purposes: 1). distinguishing KC from normal subjects; 2). identification of early KC from non-KC cases; 3). staging of KC. Overall, these algorithms demonstrated good differentiation of normal and keratoconic eyes, as well as good differentiation of normal from early KC providing a sensitivity of between 69% and 100% and specificity of between 90% and 100%. However, these studies had the limitations of small sample size and being tied to a specific imaging system. Further, none of the results have been translated to the clinic due to a lack of adequate validation and complexity of results.

Conclusions
The application of AI and machine learning is a burgeoning field for the study of early KC detection with the aim of preventing the need for corneal transplantation. Further studies with larger sample size and a wider range of parameters from advanced corneal imaging systems are required to produce globally accepted results.

P-58 DEVELOPING AN IMMUNOHISTOCHEMISTRY PANEL FOR THE DIAGNOSIS AND DIFFERENTIATION OF BASAL CELL CARCINOMA

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Introduction
Basal cell carcinoma (BCC) is the most common neoplasm of the skin. These tumours are locally invasive, slow-growing and are confined to the epidermis. BCC is characterized by nests of basaloid cell cords with peripheral palisading. There are frequent mitotic figures located throughout the nests and cell cords, and necrotic cells are common. The clinical presentation and associated symptoms are useful in the establishment of the diagnosis of BCC but not enough to make a definitive diagnosis. Hence, developing an immunohistochemistry (IHC) panel can be helpful in the differential diagnosis compared to melanoma and/or squamous cell carcinoma (SCC).

Aim
To design and develop an IHC panel for the accurate diagnosis and differentiation of BCC.

Method
Archival human patient samples were examined to confirm the presumptive diagnosis using an IHC panel. A range of control tissues were used to validate the staining quality, prior to analysis on the test tissue. The following IHC markers including BerEP4, BCL2, CK5/6 and S100 were used as part of the panel.

Results
IHC was conducted and analysed using a light microscope. The stained specimens were examined and scored on the basis of their intensity. BerEP4 and BCL2 show positivity for all BCC cases. CK5/6 appeared positive for cases presenting with SCC. Contrastingly, S100 appeared positive for cases presenting with melanoma.

Conclusion
The analysis of BerEP4, BCL2, CK5/6, S100 can be used as a helpful immunohistochemical panel in the diagnosis and differentiation of BCC.

P-59 ASSESSING THE GROWTH OF HUMAN-DERIVED OSTEOSARCOMA TUMOUR CELLS WITHIN HYDROGEL SCAFFOLDS FOR THE DEVELOPMENT OF A 3D-PRINTABLE OSTEOSARCOMA ORGANOID

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* Duchi and Onofrillo contributed equally to this work.

Introduction
Standard monolayer cell cultures have been found to produce unreliable therapeutic results due to its inability to recapitulate key elements of the tumour microenvironment. The pressing need to create more physiologically relevant preclinical models has led to the development of 3D organoids that better reflect the crucial interactions found *in-vivo* and has great potential for studying rare diseases, such as osteosarcoma (OS).
In order to achieve a deeper spatial complexity compared to previously described organoids, we propose a 3D OS model in which a cellular 'CORE' is constituted by tumour cells embedded *within* biocompatible hydrogels, surrounded by a mineralized 'SHELL' to mimic the interface with the cortical bone. However, the photocrosslinking process to solidify hydrogels needs to be fine-tuned to obtain the proper mechanical properties while limiting the cytotoxic effect derived from the generation of free radicals during the hardening process.

Aim
Aided by novel 3D printing technologies, this study aims to develop a bioprinted *ex-vivo* organoid that better mimics the OS tumour microenvironment, dubbed the '3D-OSTEO'. In this work, we present our preliminary steps towards validating the cellular 'CORE' – focusing on the viability and proliferation of tumour cells after encapsulation in a photocrosslinkable gelatin-methacryloyl (GelMA) hydrogel.

Methods
Our CORE biofabrication approach involved mixing tumour cells into GelMA to generate an evenly mixed hydrogel/cell suspension, termed the 'bioink', which was then extruded and photocrosslinked to produce a stiff cell-embedded hydrogel scaffold.
In order to demonstrate the experimental feasibility of this biofabrication approach, we delivered bioink comprising human-derived OS cell lines i) U-2 OS, ii) Saos-2 iii) 143B in GelMA 10% into customized wells, and subsequently tested its capacity for proliferation and viability, up to 14 days post-biofabrication.

Results
With 60s of photocrosslinking at a fixed light intensity and wavelength of 10mW/cm^2 and 405nm respectively, we successfully generated GelMA scaffolds bearing a compressive modulus of 50kPa, which is the reported matrix stiffness for optimal OS cell growth. CellTiter-Blue fluorescent assays showed that the hydrogel-embedded tumour cells survived the initial free radical exposure and continued to proliferate until confluency was reached by 14 days post-biofabrication. LIVE/DEAD and immunostaining analysis indicated cell aggregation, motility and extracellular matrix (ECM) remodeling.

Conclusion
Our findings establish the conditions that ensure optimal survival and proliferation of hydrogel-encapsulated cells and validates our biofabrication approach. This sets the basis for the development of a novel organoid that has the potential to revolutionize preclinical drug testing, with a positive impact for OS patients.

P-60 DRUG COMBINATION THERAPY DEVELOPMENT FOR METASTATIC COLORECTAL CANCER

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Introduction
Although targeted agents have prolonged the lives of patients with metastatic colorectal cancer (mCRC), clinical responses are limited to a subset of individuals and are generally short-lived with most tumours developing resistance within a few months. Experimental and theoretical studies of the development of resistance to targeted cancer treatment have shown that small numbers of cells resistant to any given drug are usually present in large solid tumours at the start of therapy. A solution proposed to overcome this challenge is combination therapy, with the simultaneous administration of two or more drugs that target different essential pathways.

Aims
Develop novel drug combination therapies and predictive markers for patients with metastatic colorectal cancer.

Methods
Here, we performed a screen of 919 approved, experimental and investigational agents in combination with standard-of-care chemotherapy backbones (5FU/irinotecan/oxaliplatin) in a panel of 12 colorectal cancer (CRC) cell lines. We are screening our leading combinations with patient-derived CRC organoids derived from liver or lung metastases, combining dynamic imaging and endpoint assays. DNA sequencing, Western blotting and Immunofluorescence assay are used to collect genomic data and main signalling pathway features.

Results
24 lead combinations were selected for follow-up based on their drug approval, clinical trial status and novelty. Meanwhile, 6 Patient-derived mCRC organoids (mCRC PDTOs) from liver or lung metastases were successfully cultured for drug screening. Morphological changes of these mCRC PDTOs showed different effects of 5FU, SN38 and Oxaliplatin. Distinctly different drug responses and main signalling pathway features were also observed among different mCRC PDTOs. Our mCRC PDTOs can thus capture the inter-tumoral and intra-tumoral heterogeneity of mCRC and are ideal for testing our novel drug combinations.

Conclusion
By combining drug response data, clinical data, main signalling pathway features and genomic data of mCRC organoids, we are developing novel drug combination therapies and predictive markers for mCRC patients.

P-61 KINESIN FAMILY MEMBER 1A: EVOLVING ROLE IN RETT SYNDROME

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Introduction
Rett Syndrome (RTT) is a severe neurodevelopmental disorder that is mainly caused by mutations in the methyl-CpG-binding (*MECP2*) gene; however, between 5 – 40% of RTT patients are genetically undiagnosed. Classical RTT patients undergo normal development until 6 -18 months of age after which development starts regressing and they develop characteristic abnormal hand movements which are a hallmark of classical RTT. Atypical RTT patients have many features of classical RTT but do not meet all of the specific diagnostic criteria. Using whole-genome sequencing (WGS), we have identified a novel *de novo* heterozygous missense variant (c.744C>A; p.(Asp248Glu)) in the motor domain of kinesin-3 family member 1A (*KIF1A*) in one classical RTT proband. KIF1A encodes a neuron-specific kinesin motor protein that is crucial for ATP-dependent anterograde axonal transport of key synaptic cargo.

Aims
Our aim is to study the role of *KIF1A* in the pathogenesis of RTT.

Methods
We have evaluated a number of individuals with varying clinical phenotypes who had variants in *KIF1A*. Pathogenicity of variants has been evaluated using *in silico* tools such as SIFT, MutationTaster and PolyPhen-2. The HOPE database was used for the three-dimensional (3D) protein modelling. Various functional assays, including neurite tip accumulation and the microtubule gliding assay have been undertaken to observe the effect of patient variants on KIF1A motor domain function.

Results
Through various collaborations, we have identified 18 different heterozygous *KIF1A* variants in 25 probands with many clinical features seen in RTT. All variants are localized within the highly conserved motor domain of KIF1A, with 11 variants being novel. Various *in silico* tools predict all the variants to be pathogenic and 3D protein modelling predicts all variants will disrupt correct protein folding and/or will have a deleterious effect on ATP hydrolysis rate. For 4 of the novel variants, we found that the ability of mCitrine-tagged KIF1A-motor domain proteins to accumulate in neurite tips of differentiated SH-SY5Y cells is reduced by > 90-95% ($p<0.0001$) compared to the wild type protein. In addition, the *in vitro* microtubule gliding assay showed that the velocity of rhodamine-labelled microtubules over immobilised variant KIF1A-motor domain protein is significantly reduced compared to the wild type protein. This suggests that the mutated KIF1A proteins show a significant defect in movement along cellular microtubules. Complementary studies using live-cell single-molecule KIF1A tracking and ATPase assays are underway.

Conclusions
This work has identified a potentially novel disease-causing gene in RTT/RTT-like patients, thereby improving genetic diagnosis rate.

P-62 ENHANCING TISSUE REGENERATION BY PROMOTING REGULATORY T CELL ACCUMULATION INTO DAMAGED TISSUES

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Introduction

Regulatory T cells (Tregs) are immuno-suppressive and pro-regenerative cells which play a critical role in tissue regeneration. Not only do they help to promote an anti-inflammatory environment, but they also directly act on stem/progenitor cells by expressing regenerative factors, such as morphogens. Therefore, one might induce tissue regeneration by promoting the accumulation of Tregs into damaged tissues. We identified a growth factor able to promote the accumulation of Tregs when delivered into injured tissues. Using rational protein engineering, we seek at improving the activity of the growth factor on Tregs and subsequently tissue regeneration.

Aim

First, we aim at engineering the wild-type growth factor (wt-GF) with enhanced signalling on Tregs by modulating its affinity for its main receptor. As a strategy, we substituted the receptor-binding site of the wt-GF with the receptor binding site of a high-affinity ligand (Hi-GF) to generate engineered variants (E-GFs). The activity of E-GFs on Tregs will be tested *in vitro* and in tissue regeneration models in the mouse.

Methods

Different variants of E-GFs were designed by partial substitution of the wt-GF receptor binding site with the Hi-GF receptor binding site. E-GFs binding affinity was tested by a direct binding assay; its effect on Tregs through migration assay on mouse spleen Tregs; the triggered receptor internalization through receptor internalization/degradation assay on HaCaT cells. The most promising E-GFs variant was tested i*n vivo*, in mouse skin, muscle and bone tissues through local delivery of E-GF in a fibrin hydrogel. We then assessed the Tregs accumulation through flow cytometry as well as the efficiency of E-GF to promote bone regeneration in the mouse.

Results

We show that one E-GF variant has a higher binding affinity for the receptor and induced an enhanced Tregs migration compared to wt-GF, while inducing slightly lower receptor internalization/degradation than Hi-GF. Then, preliminary *in vivo* results show that local delivery of E-GF on mouse skin, muscle and bone injuries promoted wound closure and Tregs accumulation.

Conclusion

This study shows that promoting Treg accumulation at a wound site via an engineered GF promotes tissue regeneration. The engineered GF is able to regulate both the immune response and regeneration after tissue damage. Modulating the Tregs activity is a promising new strategy for regenerative medicine applications.

P-63 TARGETING NEUROINFLAMMATION IN PARKINSON'S DISEASE

★ ***BML travel award***

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Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, resulting in the loss of dopamine in the striatum. Current dopamine-replacement therapies for PD provide only symptomatic relief and are not known to alter disease progression. It is well known that inflammation is involved in the initiation and progression of PD. Dopamine-releasing neurons are particularly vulnerable to inflammatory attack and it has been shown that microglia, are toxic towards dopamine-releasing neurons in culture and in animal models of PD. The dopamine D3 receptor (D3R) is expressed in neurons, glia, including microglia, and infiltrating immune cells. Blockade of D3Rs has been shown to elicit potent therapeutic effects in animal models of PD.

Aims

This project aims to demonstrate that our drug treatment improves locomotor impairment and reduce anxiety in animal models of PD. To dissect the specific contribution of dopamine D3 receptors, specifically in microglia, in the neuroprotective and anti-inflammatory effects of drug treatment.

Methods

This will be explored by utilising the rotenone model of PD as a platform to modify the underlying progression of PD, by repurposing an existing drug to modulate neuroinflammation and thereby limit degeneration. We will perform behavioural (open field test) and clinical assessments throughout our protocol. Upon completion of our 21-day paradigm, we will harvest tissue to perform several biochemical and histological analyses to determine the effect of our drug on dopaminergic cell loss, gliosis, microgliosis, cell death, inflammatory profiles, axonal integrity and degeneration. We will also utilise BV-2 microglial and SH-SY5Y neuronal cell lines to identify the specific signalling pathways activated by our drug treatment.

Results

We have shown that microglia activation results in perturbed dopamine receptor expression. Specifically, D2R, D3R and D5R levels were significantly reduced ($p<0.01$, *t*-test) after 24h exposure to 1ug/ml lipopolysaccharide (LPS). These perturbations are likely to be homeostatic mechanisms to regulate cell function in the presence of inflammation. Other studies have shown D3R specificity of our drug in other disease contexts like substance abuse disorder, alcohol addiction and schizophrenia.

Conclusions

From our preliminary studies and the literature, we expect to show that our drug will ameliorate *in vivo* rotenone-induced PD and anxiety through the specific involvement of the D3R in reducing microglial activation, hence chronic inflammation and cell loss in the substantia nigra pars compacta and striatum.

P-64 TEMPORAL BONE SURGICAL SKILL TRANSFER FROM VIRTUAL REALITY TO 3D PRINTED BONES

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Introduction

Surgical training has traditionally relied on cadaveric dissection and supervised practice in the operating room. With the advancement of technology, surgical training using haptic enabled virtual reality (VR) has become a viable option to overcome the disadvantages of traditional training, such as resource scarcity. Literature has shown that surgical skills improve after VR training. However, the transferability of those skills from VR to real-life settings is not properly addressed.

Aims

This study investigates the transfer of surgical skills learned in VR to a physical medium, namely, 3D printed specimens, in the domain of temporal bone surgery.

Methods

The University of Melbourne temporal bone surgery simulator was used in this study. This simulator consisted of 3D rendered temporal bone cases generated from micro-computer tomography (CT) scans of cadaveric temporal bones, a haptic device that provided tactile feedback and a MIDI controller for changing settings. The same temporal bone models were used to produce 3D printed temporal bones.

Twenty novice students who were interested in surgery from Victorian Universities were recruited to participate for two consecutive days. Participants watched a video tutorial on how to perform a simple temporal bone surgery: cortical mastoidectomy. After familiarization with the simulator, they were trained on the simulator on four different temporal bones (bones 0, 1, 2, and 3). Each case was repeated twice, the first with feedback and the second without feedback. Then, a pre-test was conducted with participants performing the surgery on a different temporal bone (bone 4) without feedback. Next, they performed the surgery on two 3D printed temporal bones (bones 4 and 5), which were recorded as post-tests 1 and 2 respectively.

The pre and post-test performances were evaluated by two blinded ENT surgeons using an assessment scale specifically designed for cortical mastoidectomy (out of 20). The scores were analysed using a one-tailed t-test.

Results

The difference between the mean of the post-test and pre-test scores were calculated to indicate the performance difference from VR to 3D media. This difference was normally distributed, as assessed by Shapiro Wilk test ($p>0.05$). The difference of the mean of post-test and pre-test was 1.30 (SD=2.88), indicating a significant positive performance difference, $t(19)=2.024$, $p=0.029$ (one-tailed).

Conclusion

The results of this study showed that there was significant positive skill transfer from VR and 3D printed bones. Therefore, VR surgical training is an effective alternative to lessen cadaver usage.

P-65 BMP4 INHIBITS BREAST CANCER METASTASIS INDEPENDENT OF TUMOUR SMAD4

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Introduction

Breast cancer affects 1 in 8 women worldwide. Localised breast cancer is largely curable, however, due to a lack of effective therapies, patients with tumours that have spread (metastasised) have a poor 5-year survival rate at 27%. In our previous search for potential therapeutic targets that modulate metastasis, an anti-metastatic protein called bone morphogenetic protein 4 (BMP4) was identified. Compared to localised breast tumours, metastatic tumours express lower levels of BMP4. By ectopically restoring BMP4 expression in tumours, metastasis can be suppressed in mice.

However, BMP4 was reported to promote tumour progression in colorectal and pancreatic cancers. We noted that a critical mediator of BMP4 signalling, SMAD4 (mothers against decapentaplegic 4), is mutated or lost in up to 40% of these cancers, rendering canonical BMP4 signalling defective. It has also been reported that in the absence of SMAD4, BMP4 induces non-canonical signalling through NF-κB, PI3K/AKT and MAPK pathways.

Aims

We investigated whether the anti-metastatic effect of BMP4 is dependent on SMAD4, and whether the detrimental effects of BMP4 in cancers without functional SMAD4 arise from activation of non-canonical signalling.

Methods

In human breast cancer MDA-MB-231-HM cells, SMAD4 was reduced with short hairpin RNA. In SMAD4-null MDA-MB-468 cells, SMAD4 was ectopically restored. BMP4 was then ectopically expressed in these metastatic lines. Orthotopic tumours were established via injection into the 4th mammary fat pad of NSG mice. Tumours were monitored and resected at 400 mm^3, and subsequent development of metastasis was characterised.

Results and conclusions

Loss of SMAD4 abrogated canonical BMP4 signalling based on attenuation of BMP4 target genes (Id1/2 and Smad7; $P<0.05$). Consistent with our hypothesis, BMP4 accelerated the growth of tumours with low or no SMAD4 ($P<0.05$) potentially due to increased angiogenesis, while having no effect on those that expressed SMAD4. This suggests that BMP4 agonists are not appropriate therapy options for patients who have SMAD4-low tumours. Surprisingly, BMP4 significantly inhibited metastasis regardless of SMAD4 expression in tumours ($P<0.01$), indicating that downstream signalling can be targeted to reduce cancer spread.

We are currently investigating mediators of this anti-metastatic effect by RNA sequencing and mass spectrometry. We are also testing small molecules that activate BMP4 signalling as a potential therapy to treat metastatic breast cancer that expresses SMAD4, and to improve patient survival.

P-66 KNOWLEDGE, ATTITUDE AND PRACTICE OF FOOT AND MOUTH DISEASE AMONGST PASTORAL PRACTITIONERS ADJOINING THE BANNERGHATTA NATIONAL PARK

★ ***BML travel award***

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Introduction

Foot and Mouth Disease (FMD) is a vaccine-preventable viral infectious disease caused by Foot and Mouth Disease Virus manifesting in cloven-footed animals of order *Artiodactyl* (domestic livestock and animals.) The epidemiological complexity added by characteristics such as high transmissibility rate exaggerate challenges in control of the disease. Moreover, the presence of around 300 pastoral villages adjoin the protected area of Bannerghatta National Park (BNP),Karnataka, provides an opportunity for "spillover". Due to the high dependency on the livestock for a livelihood, the economic burden of FMD is very high. Successful livestock disease program requires understanding the knowledge, attitude and practice of the livestock owners and their readiness to follow the implementation of these measures.

Aim

To assess knowledge, attitude and practice (KAP) among livestock owners at ecotone villages in BNP.

Methodology

A cross-sectional descriptive survey was conducted at 12 villages adjoining BNP to assess the KAP about FMD amongst pastoral practitioners. The data was collected using a validated KAP questionnaire among 83 households comprising of 206 interviewees. The questionnaire consisted of four sections, each section addressing socio-economic details, questions to assess knowledge, attitude and practice, respectively. Scoring was done on a binary basis (0 or 1), followed by statistical analysis.

Result

It was observed that although 92.23% had 'ancestrally bequeathed' knowledge about the incidence and prevalence of the disease. The overall KAP score was considered to be average. There seemed to be major misconceptions about vaccination schedule for FMD, especially, neonatal vaccinations and revaccination schedules in livestock. Importantly, irrespective of pre-exposure prophylaxis to FMD, 75.24% participants reported of recent FMD outbreak in their herd with almost 57.28% of them occurring between 2018-19. A strong association was seen between the lack of education and experience in livestock with FMD occurrence. The practice of outdoor grazing in or near forest was a prevalent practice.

Conclusion

The study revealed that immediate action and interventional activities for betterment in practices for the prevention of infectious diseases was required. The list of "hot- spot" villages will be submitted to the Dept. of Forestry for further actions. Also, recurrence of FMD despite regular vaccination points towards serious dilemmas with regard to the efficacy of vaccination and its availability. The community members were positive about preventive care and disease monitoring if they received support from the responsible stakeholders. Limitations of the study included relatively less sample size and future prospective includes sero-surveillance of the area for correlation with epidemiological data.

P-67 ROLE OF FANCM IN GENOME STABILITY AND FERTILITY IN MICE

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Introduction
Fanconi anaemia (FA) is a rare genetic disease characterised by life-threatening anaemia and reduced fertility. The mechanism underlying reduced fertility in FA patients is not understood, however, it is known that meiosis is essential for fertility to ensure correct chromosome segregation. FANCM, a translocase protein part of the FA pathway, binds to DNA branched structures (e.g. stalled replication forks), which are especially prevalent during S-phase replication and meiotic crossovers. FANCM recognises and binds to these DNA branched structures with high affinity to maintain genome stability and initiate DNA damage repair. FANCM mutations have previously been shown to play a role in fertility and meiosis in *Arabidopsis thaliana*. My project aims to understand this process in mammals.

Aims
1) To assess the role of FANCM in genome stability in mice.
2) Use *Fancm*-deficient mice to investigate the role of FANCM in fertility and meiosis.

Methods
1a) Murine B cells are isolated and cultured for 4 days before treating with DNA damaging agents (hydroxyurea and mitomycin C) to investigate how *Fancm* B cells respond to different types of replication stress.
1b) Murine B cells are also incubated with CellTrace™ Violet and treated with LPS to stimulate proliferation. Flow cytometry analysis was undertaken to examine proliferation rates.

2a) Male *Fancm*$^{-/-}$ mice of 6-10 weeks of age were sacrificed and the testes were harvested to perform a full fertility analysis. Testis and epididymis were weighed. Daily sperm production and sperm motility were assessed.
2b) We have *Fancm* mouse models on two different genetic backgrounds, enabling linkage maps analysis to measure recombination by sequencing single nucleotide polymorphisms (SNPs) that are unique to each strain. Immunofluorescent chromosome meiotic slides were also performed to investigate how meiosis-specific proteins play a role with FANCM.

Results
Fancm-deficient B cells treated with DNA damaging agents show altered expression of certain proteins involved in the DNA damage response. Proliferation rates in the B cells were not significantly different between control and mutant mice.

Fancm$^{-/-}$ mice are not significantly different in body weight but have reduced testis ($p<0.0001$) and epididymis ($p<0.01$) weight. We also observed decreased sperm motility ($p<0.01$), progressivity ($p<0.01$) and reduced daily sperm production ($p<0.05$). From our linkage studies, we detected an increase in recombination in *Fancm*$^{-/-}$ mice, suggesting that the reduced fertility could be due to a hyper-recombination phenotype.

Conclusion
Homozygous mutations in FANCM have been shown to cause infertility in patients. Our data suggest that FANCM could have a role in meiosis that affects the fertility in our mouse model. Our work will provide insight into how FANCM can maintain genome stability in a mouse model.

P-68 CHARACTERISING THE PATHOGENICITY OF A NOVEL COLORECTAL CANCER GENE CANDIDATE

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INTRODUCTION

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer in Australia. Despite improvements in treatment, CRC remains the 2nd leading cause of cancer mortality. There's an urgent need to better understand CRC etiology and progression to develop more effective therapies. Genome sequencing projects have revealed the landscape of alterations found in CRC, highlighting multiple novel candidate driver genes. Our cancer gene candidate, on chromosome 4q, was identified as recurrently deleted in CRC, however the contribution of its loss to cancer development is unknown.

AIMS

To determine the prevalence, mechanisms and biological consequences of the loss of our cancer gene candidate in human colorectal cancer.

METHODS

To define the prevalence of our cancer gene candidate's loss in CRC, copy number alteration studies were performed using CRC cell line data, inhouse patient samples and data from the TCGA. Expression of mRNA and protein were assessed in CRC primary specimens and cell lines. Wild-type (WT), heterozygous knock-out (KO) and homozygous KO cell lines have been generated using CRISPR/Cas9 technology and validated using Sanger sequencing and Western blot. Phenotypic and functional consequences of loss of our cancer gene candidate have been evaluated in relation to viability, clonogenic potential, proliferation, cell cycle, death and drug responses.

RESULTS

DNA copy-number alteration studies indicate deletions of our cancer gene candidate occur in ~10% of CRCs. Point mutations are rare, however, preliminary mRNA and protein expression data indicate loss of expression in up to 20% of wild-type tumours. Compared to the WT cell lines, the heterozygous and homozygous KO cells exhibited increased cell viability and elevated clonogenic potential. Cell cycle analyses revealed significantly greater populations of KO cells compared to WT cells in G2/M phase. Heterozygous and homozygous KO cells also exhibit increased resistance to treatment with BH3 mimetic drugs *in vitro*.

CONCLUSIONS

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

P-69 EPITHELIAL PROTEASE-ACTIVATED RECEPTOR 2 ACTIVATION LINKS CELLULAR METABOLIC CHANGES TO INNATE IMMUNE RESPONSES

★ ***ExoPharm travel award***

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Introduction

The consensus view of mammalian immunity is that specialised immune cells primarily organize the host defence against infectious and physiological stresses to maintain homeostasis. However, this perception ignores a growing realization that other cell types have an innate capacity to defend themselves against stress-induced disrupted homeostasis. Epithelial cells play important roles in barrier defence, but their ability to autonomously respond to chemical, oxidative or mechanical stress and maintain tissue integrity is not well appreciated.

Aims

Protease activated receptor 2 (PAR2), one of four membrane-spanning G-protein coupled receptors in the PAR family, is activated by multiple inflammatory serine proteases and specific ligands, linking proteases/ligands outside cells to signalling pathways inside cells. However, no stimuli have been reported yet to induce immune responses linked to glycolysis in epithelial cells. In our study, we investigated the mechanisms of metabolic control by PAR2 of the immune response, and the impacts of metabolism and inflammation on epithelial cells.

Methods

Various endogenous proteases, PAR2 ligands and pharmacological inhibitors targeting different G-protein signalling that were used to investigate the intracellular signalling pathways in both *in vitro* and *in vivo* studies.

Results

Here we show that certain endogenous damage-associated proteases that act on PAR2 can reprogram epithelial cell metabolism for glycolysis to fuel sterile inflammation. Stimulating PAR2 on the cell surface increases glucose uptake and switches cell metabolism from oxidative phosphorylation towards aerobic glycolysis and lactate production. Further, this PAR2-driven glycolysis and an associated inflammatory defence signature including increased expression of IL25, IL33, IL1α, IL1β can be suppressed by a PAR2 antagonist or by the glycolysis inhibitor, 2-deoxy-D-glucose.

Conclusions

Mechanistically, PAR2-mediated G_q-$G_{\beta\gamma}$-PI3K_γ-Akt-mTOR1 stress-signalling axis is revealed here as a missing link in autonomous maintenance of cellular homeostasis and represents a new mechanism for protection of host tissues from the environment. While epithelial PAR2 can help to maintain cellular homeostasis and tissue integrity, its over-stimulation may lead to inflammatory or metabolic diseases where PAR2 may be a therapeutic target.

P-70 LOOKING TOWARDS GENE THERAPY: CHARACTERISATION OF EX VIVO AAV2.CYP4V2 GENE THERAPY IN HUMAN RETINAL EXPLANTS

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Introduction
Bietti's crystalline dystrophy (BCD) is an incurable inherited retinal disease (IRD) caused by mutations in the *CYP4V2* gene that results in progressive degeneration of the retinal pigment epithelium and photoreceptors, leading to blindness by middle age. Clinical trials investigating adeno-associated virus (AAV)-mediated gene delivery to the retina for other IRDs have reported compelling data on safety and efficacy. Pre-clinical cell culture studies are required to assess the *in vitro* efficacy of a viral expression system. Human retinal explants collected during clinically indicated retinal detachment repair from live donors is a relatively new technique for validation of gene therapy constructs. It uses tissue that would otherwise be discarded and requires no change to standard surgical techniques.

Aims
1) To utilise human retinal explants obtained during routine vitreoretinal surgery to investigate the efficacy of AAV2.CYP4V2 transduction in an *ex vivo* model. 2) To investigate human retinal explants as a viable collaborative technique between the Royal Victorian Eye and Ear Hospital and the Centre for Eye Research Australia.

Human retinal explants were collected during surgery from consenting patients. Explants of at least 0.5 mm diameter were cultured and transduced with 1.43 x 10^{10} vg of AAV2.CYP4V2, or left untransduced as a negative control. After 7 days, explants were fixed, frozen and cryosectioned. Immunohistochemistry was performed using immunolabels for CYP4V2 and retinal cell markers (rhodopsin, calbindin, protein kinase C alpha) to assess toxicity and transduction efficacy in the human retina.

Results
Immunolabelling of cultured human retinal explants transduced with AAV2.CYP4V2 demonstrated robust expression of CYP4V2 in retinal cells, which was absent in the untransduced controls. Transduced retina did not show clear signs of AAV-mediated toxicity.

Conclusion
These pilot data demonstrate viral-mediated expression of a *CYP4V2* transgene in human retinal tissue and support the further development of gene therapy for BCD, a progressive blinding disease. Human retinal explants are a valuable model that can be used for *in vitro* validation of AAV gene delivery into human retinal cells.

P-71 NO MORE MR. FUN GUY: IN VITRO MODELLING OF CANDIDA ALBICANS-INDUCED DEATH IN HUMAN MACROPHAGES

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Introduction

Candida albicans is the most common fungal causative agent of life-threatening infections in immunocompromised patients, with mortality rates of invasive disease as high as 50%. Macrophages are an important first line of defence against *C. albicans* infection. In vitro experiments with mouse macrophages suggest that *C. albicans* escapes macrophage phagocytosis by forming hyphae and triggering a pro-inflammatory form of cell death known as pyroptosis. Escaped *C. albicans* hyphae rapidly deplete glucose, without which activated macrophages cannot survive.

Aims

In this project, we aim to investigate the impact of NRLP3 inflammasome activation and glucose homeostasis on *C. albicans*- macrophage interactions. Our goal was to compare the outcomes of *C. albicans* live-cell infections for THP-1 cells, human monocyte-derived macrophages (hMDMs), and mouse primary bone-marrow-derived macrophages (mBMDMs).

Methods

In this project, we use live-cell microscopy to characterize interactions between *C. albicans* and macrophages at distinct stages of infection, namely phagocytosis*, C. albicans* escape and macrophage pyroptotic and glucose-dependant death. Fluorescence activated cell sorting (FACS) was used to establish the percentage of $CD14^+$ monocytes isolated from human blood.

Conclusions

Consistent with previous findings in mBMDMs, biphasic death is seen following *C. albicans* phagocytosis in THP-1 cells (a human monocytic cell line). However, a NLRP3 inflammasome inhibitor (MCC950) only partially inhibited *C. albicans*-induced phase I death in THP-1 cells. Diminished phase I death was observed for hyphal-deficient efg1Δ/Δ cph1Δ/Δ *C. albicans* mutant infection of THP-1 cells, demonstrating that hyphal formation is crucial for phase I killing. In human peripheral blood mononuclear cell (PBMC)- derived macrophages we observe a high phagocytosis rate, and good hyphal formation by *C. albicans* once phagocytosed. Despite this, preliminary findings suggest that the NLRP3-inflammasome mediated death is not observed for all donors. Overall our findings highlight differences in *C. albicans*-induced NRLP3 inflammasome mediated death in mBMDMs, THP-1s and hMDMs.

P-72 RETINOIC ACID RECEPTOR γ ACTIVITY IN ENDOTHELIAL CELLS REGULATES HAEMATOPOIESIS AND BONE MARROW NICHE INTEGRITY

Diannita Kwang[1,2], Kelli Schleibs[1], Lenny Straszkowski[1], Ashleigh King[1,2], Jörg Heierhorst[1,2], Gavin Tjin[1], Louise E. Purton[1,2]

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Introduction
All-trans retinoic acid, the biologically active form of vitamin A, has been shown to be a major regulator of haematopoiesis. We have previously shown that mice null for the vitamin A receptor, retinoic acid receptor γ (*Rarg*$^{-/-}$ mice), developed a myeloproliferative-like syndrome, accompanied by reduced bone marrow (BM) B lymphocyte and erythrocyte subsets, and these phenotypes were shown to be microenvironment-induced. Further investigations have identified that the deletion of *Rarg* in distinct mesenchymal lineage cell types resulted in B lymphopoiesis defects. However, these defects did not fully recapitulate the haematopoietic phenotypes observed in *Rarg*$^{-/-}$ mice.

Aims
To determine the effect of loss of *Rarg* in endothelial cells (ECs) on haematopoiesis and blood vessel niches.

Methods
We deleted *Rarg* in ECs in 3-week old mice and investigated their haematopoiesis and BM niche parameters at 12 weeks of age. All mice were crossed to *Rosa26*mTmG reporter mice which confirmed that *Rarg* deletion was restricted to the ECs.

Results
Male and female ECCre:*Rarg*$^{\Delta/\Delta}$mTmG mice had reductions of B lymphocytes in their peripheral blood (PB), accompanied by significant reductions in BM mature recirculating B cells compared to sex-matched ECCre:*Rarg*$^{+/+}$mTmG mice. Interestingly, no significant phenotypes were observed in the maturing B lymphocyte populations in the BM or spleen of female mice, whereas male mice had reduced numbers of PreproB and increased numbers of PreB lymphocytes in their BM. Female ECCre:*Rarg*$^{\Delta/\Delta}$mTmG mice also developed PB thrombocytopenia and macrocytic anaemia, accompanied by increased numbers of erythrocytes in the spleen. Male ECCre:*Rarg*$^{\Delta/\Delta}$mTmG mice had significantly reduced numbers of mature BM erythrocytes. The BM sinusoidal vessels in female, but not male ECCre:*Rarg*$^{\Delta/\Delta}$mTmG mice were significantly dilated, and these female mice also had smaller adipocytes in their BM.

Conclusions
Collectively, our data showed that RARγ activity in endothelial cells is important for B lymphopoiesis, platelet production and erythropoiesis in male and female mice. Furthermore, RARγ activity in endothelial cells is essential for the regulation of sinusoidal vasculature structure and adipocyte production in female mice. Further investigation into the molecular mechanisms are important and may reveal novel therapies for a range of haematopoietic diseases involving B lymphocytes, erythrocytes and platelets. Vitamin A deficiency results in anaemia and has significant, life-threatening effects due to impaired immune cell number and function. This study shows that many of these effects are due to altered microenvironment cell support of haematopoietic cells, rather than being intrinsic to the haematopoietic cell.

P-73 HOXA1-OVEREXPRESSION IN SERIAL MURINE BONE MARROW TRANSPLANTS INDUCES THROMBOCYTOPENIA AND HAEMATOPOIETIC STEM CELL MYELOID-PRIMING

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Introduction
Myelodysplastic syndromes (MDS) are heterogeneous haematopoietic myeloid malignancies diagnosed in approximately 1200 Australian patients per annum, 30% of whom progress to secondary acute myeloid leukaemia (sAML). Elucidating the mechanisms underlying MDS and thereby developing targeted therapeutics has been hindered by disease heterogeneity and the lack of mouse models that accurately reflect the human disease. Computational gene expression analysis of immature bone marrow (BM) cells from MDS patients revealed that *HOMEOBOX A1* (*HOXA1)*, a transcription factor, is increased in 50% of MDS patients. This is restricted to the full-length (*HOXA1-FL)* isoform, with normal truncated (*HOXA1-T*) isoform expression. Conditional knock-in overexpression mouse models were generated utilising two constructs: *WT-Hoxa1* (expresses both isoforms) and *MUT-Hoxa1* (expresses only *Hoxa1-FL*) based on the hypothesis that *Hoxa1* plays a dose-dependent role in MDS and leukaemia development.

Aim
To determine whether haematopoietic stem cell (HSC)-overexpression of *Hoxa1* in serial whole BM transplant (BMT) is sufficient to induce MDS and sAML.

Methods
Whole BM was isolated from 8-13wk female conditional knock-in hScl-Cre-ER^{T} *WT-Hoxa1*$^{fl/+}$, *WT-Hoxa1*$^{fl/fl}$, *MUT-Hoxa1*$^{fl/+}$, *MUT-Hoxa1* $^{fl/fl}$ and Cre+$^{+/+}$ mice and 5x10^{6} cells intravenously injected into 8-11wk male lethally-irradiated primary recipients. *Hoxa1*-overexpression in HSCs was induced via tamoxifen chow provision. Peripheral blood (PB) and BM parameters were analysed via automated haematological cell counts and flow cytometry 3.5-7months post-BMT and secondary BMTs performed at 6-7months. Data were pooled from two experimental cohorts, total n=8-16 per genotype.

Results
Profound PB thrombocytopenia (low platelet count) accompanied by increased megakaryocyte progenitors was observed in primary and secondary *WT-Hoxa1*$^{fl/fl}$ and *MUT-Hoxa1*$^{fl/fl}$ recipients. *MUT-Hoxa1*$^{fl/fl}$ recipients exhibited normal PB red blood cells (RBC) but substantially reduced BM RBC and erythroid cells, particularly those undergoing late erythropoiesis. Secondary *MUT-Hoxa1*$^{fl/fl}$ BMTs also exhibited splenomegaly. Additionally, all *MUT-Hoxa1*$^{fl/fl}$ recipients presented with increased PB granulocytes, accompanied by elevated immature BM granulocytes in secondary BMTs. HSC and immature progenitor populations, especially multipotent progenitors, were reduced in all Hoxa1 BMTs in conjunction with increased common myeloid progenitors in *MUT-Hoxa1*$^{fl/fl}$ recipients.

Conclusions
Overexpression of *Hoxa1* in murine HSCs causes profound thrombocytopenia, dyserythropoiesis and HSC myeloid-priming but is insufficient to induce sAML in primary or secondary BMTs. Phenotype severity is dose- and isoform-dependent with heterozygous recipients and *WT-Hoxa1* BMT recipients exhibiting less severe phenotypes. Further analysis of the mechanisms underlying the observed phenotypes is required to elucidate the mechanisms of MDS development by HOXA1, permitting the investigation of novel therapeutics intent on reducing MDS disease burden.

P-74 SHOCK ABSORBING ABILITY IN HEALTHY AND DAMAGED CARTILAGE-BONE UNDER HIGH-RATE COMPRESSION

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Introduction

Disruption of cartilage structure, caused by injury, trauma or disorder such as osteoarthritis (OA), can alter the mechanism of load transfer from the cartilage to the underlying bone. Changes in the cartilage structure can also alter the ability of cartilage-bone to absorb and dissipate the impact energy. We aimed to compare the mechanical properties (stiffness, strain, absorbed and dissipated energies) of the cartilage-bone unit before and after modifying the cartilage by removing the top 50% of the total cartilage thickness to mimic the cartilage thickness in a grade III cartilage lesion.

Methods

Cartilage-bone specimens (n = 12, diameter = 6.52±0.09 mm and height = 8.39±1.64 mm) were harvested from the femur of a young bovine. To modify the cartilage-bone specimens, the top 50% of cartilage thickness (consisting of both the superficial tangential zone (STZ) and part of the middle zone of the cartilage) was removed. unconfined compression at a high-rate (4% strain at 5Hz) was applied to cartilage-bone before and after modifying the thickness. High-speed camera and microscope were used to capture microscopic deformation, and digital image correlation technique (DIC) was employed to quantify the deformation of cartilage and bone. The mechanical properties of cartilage and bone were calculated before and after the removal of the top 50% of the cartilage thickness.

Results and Discussion

The overall stiffness of the cartilage-bone unit decreased by 34% after the removal of the top 50% cartilage thickness. Using the DIC technique, we found that the stiffness of cartilage reduced by 39.34 %, but no significant difference was noticed in the stiffness of the subchondral bone after the removal of top 50% cartilage thickness. The ratio of intact cartilage to bone strain at 7 MPa was 28.27, which increased to 44.01 after the removal of the top 50 % cartilage. However, the strain in the bone remained unchanged after the removal of the top half cartilage thickness (z).

Conclusions

The results from this study provided insight into the altered mechanical performance of the cartilage, the underlying subchondral bone and the cartilage-bone unit before and after the removal of 50% of cartilage thickness. It was found after the modification, higher strains were recorded in the cartilage and underlying bone when compared to the intact cartilage-bone unit. However, at 7MPa contact pressure representing a normal physiological loading condition, although the modified cartilage recorded a significant increase in strain, the underlying bone remained unaffected.

Judges and Reviewers

The Biomed Link 2019 Organising Committee blindly reviewed all abstracts submitted to Biomed Link 2019.

The Biomed Link 2019 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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