ST VINCENT'S CAMPUS

POST DOC & RESEARCH SYMPOSIUM

13 – 14 SEPTEMBER 2018

CONTEMPLATE INVESTIGATE TRANSLATE

DIGITAL ABSTRACT BOOK

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POST DOC DEVELOPMENT COMMITTEE

Research and Post Doc Symposium 2018
13 September – 14 September 2018

7th Annual St Vincent's Campus Post Doc Symposium
Thursday 13th September 2018

26th Annual St Vincent's Campus Research Symposium
Friday 14th September 2018

Welcome!

The St Vincent's Research Symposium has been held annually for over 25 years and brings together the brightest minds across the Campus to share their research with peers. This event is now held over 2 days and includes the Post Doc symposium. It involves a great coming together of prominent healthcare researchers from across the St Vincent's Research Campus and our academic partners. This is an outstanding opportunity for campus staff and visitors to celebrate some of the country's finest medical research and healthcare innovation. The program will include distinguished invited speakers as well as recognising our up and coming early career researchers across the campus in our 'Rising Stars' session.

26th St Vincent's Campus Symposium Organising Committee

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9:30-9:45 am	**Liz Caldon (TKCC/Garvan).** *Cyclin E2 promotes, but cyclin E1 opposes, genome instability via rereplication in cancer cells.*
9:45-10:30 am	**PLENARY: Dr Andy Philp (Garvan).** *Live strong and prosper – the role of skeletal muscle in healthy ageing.*
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11:14-11:21 am	**Hartmut Cuny (VCCRI).** *NAD deficiency induced by gene-environment interactions as a cause of congenital malformation.*
11:21-11:28 am	**Christopher Stanley (VCCRI).** *Singlet molecular oxygen regulates vascular tone and blood pressure in inflammation.*
11:28-11:35 am	**Melissa Mangala (VCCRI).** *Investigating population variability using high throughput electrophysiological phenotyping of human induced pluripotent stem cell-derived cardiomyocytes.*
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2:00-2:15pm **Aude Dorison (VCCRI).** *Unravelling the fate of cardiac Pdgfra+ cells in healthy and injured mouse hearts using single cell RNA-seq.*

2:15-2:30pm **Dana Bliuc (Garvan).** *Cognitive decline is associated with an accelerated rate of bone loss and increased fracture risk in elderly population.*

2:30-2:45pm **Dimuthu Alankarage (VCCRI).** *Identification of clinically actionable variants from genome sequencing of families with CHD.*

Afternoon tea **2:45-3:15 pm**

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3:15-3:45 pm **Oral Talks (2x15min) Chair: Dr Sandy Stayte**

3:15-3:30pm **Simon Junankar (TKCC/Garvan).** *DNA barcoding demonstrates immuno-editing of metastatic breast cancer cells at the clonal level.*

3:30-3:45pm **Ralph Patrick (VCCRI).** *Decoding the identity and flux of cardiac cells in injury and homeostasis at single-cell resolution.*

3:45-4:45 pm **PLENARY: Dr Joanne Reed (Garvan, Young Garvan Award Winner 2017).** *Age-associated B cells: the ABC's of autoimmune disease.*

4:45-5:00pm **Closing Remarks and Prizes**

Drinks reception 5:00pm onwards

Program 26th Annual St Vincent's Campus Research Symposium *14th September 2018*

Registration *8:00-8:45 am*

Session 1 *8:45- 10:20 am*

8:45-9:00 am **Opening: Prof Terry Campbell**

9:00-9:50 am **Oral Talks (5×10 min) Chair: Dr John Zaunders**

9:00-9:10 am **Mitchell Starr (AMR)**. *An innovative HIV testing initiative: the NSW dried blood spot self-sampling HIV testing pilot program.*

9:10-9:20 am **Michelle Isaacs (SVHA)**. *Retrospective review of 64 patients with amiodarone-induced thyrotoxicosis.*

9:20-9:30 am **Amanda Hui Min Hor (Garvan)**. *Gastric emptying rate and the safety of GLP-1 receptor agonists in Prader-Willi syndrome.*

9:30-9:40 am **Sarah Alexandrou (TKCC/Garvan)**. *Mechanisms underpinning resistance to CDK4/6 inhibition in ER+ breast cancer.*

9:40-9:50 am **Oliver Skinner (Garvan)**. *Lack of protein prenylation promotes the assembly of NLRP3-dependent inflammasomes in a cell culture model of mevalonate kinase deficiency (MKD).*

9:50-10:20 am **PLENARY: Prof Geraint Rogers** (South Australian Health & Medical Research Institute)
The influence of the human microbiome on healthy ageing.

Morning Tea **10:20-11:00 am**

Session 2 *11:00 am-12:30 pm*

11:00-11:50 am **Oral Talks (5x10min) Chair: Dr David Herrmann**

11:00-11:10 am **Thao Phuong Ho-Le (Garvan)**. *Post-Fracture Mortality: A latent class analysis of multimorbidities.*

11:10-11:20 am **Angela Sheu (Garvan)**. *Visceral fat and insulin resistance is associated with lower bone turnover.*

11:20-11:30 am **Siobhan Loughnan (SVHA)**. *Gaining 'MUMENTUM': two randomised controlled trials evaluating brief internet-delivered cognitive behavioural therapy for perinatal anxiety and depression.*

11:30-11:40 am **Sunny Wu (Garvan)**. *Landscape of the breast cancer tumour microenvironment using single-cell RNA sequencing.*

11:40-11:50 am **Maria Findeisen (Garvan)**. *The acute glucose lowering effect of IC7Fc is dependent upon functional pancreatic secretion.*

11:50-12:30 pm **Fast Forward Session (12×3 min) Chair: Dr Thomas Cox**

11:50-11:53 am **Alexander Viardot (SVHA)**. Diabetes outreach clinic for the homeless: Experience and outcomes over a 3 years period.

11:53-11:56 am **Kendelle Murphy (TKCC/Garvan)**. Personalised medicine approach in pancreatic cancer reveals fine-tuned stromal FAK manipulation improves global response to gemcitabine and Abraxane, while sensitising circulating tumour cells to shear stress in transit.

11:56-11:59 am **Ha Mai (Garvan)**. Fractures and fracture-associated mortality attributable to low bone mineral density and advancing age: a time-variant analysis.

11:59-12:02 pm **Kevin Hendrawan (AMR)**. CD39+ T regulatory cell reconstitution in Multiple Sclerosis patients undergoing autologous haematopoietic stem cell transplantation.

12:02-12:05 pm **Krista Siefried (SVHA)**. Hospitalisations over a year of follow-up in adults living with HIV.

12:05-12:08 pm **John Zaunders (AMR)**. Mapping the heterogeneity of CCR5+ CD4 T cells by high dimensional flow cytometry.

12:08-12:11 pm **Ann-Kristin Altekoester (VCCRI)**. Functional analysis of a novel cardiac specific lncRNA.

12:11-12:14 pm **Joel Lasschuit (Garvan)**. Reliability of Calcaneal Quantitative Ultrasound: a prelude to use in acute Charcot neuropathic osteoarthropathy.

12:14-12:17 pm **Jennifer Massey (SVHA)**. Immune reconstitution following autologous haematopoietic stem cell transplantation for multiple sclerosis is driven by sustained thymic reactivation.

12:17-12:20 pm **Margaret Mouat (VCCRI)**. Cardiovascular health of young and aged mice lacking the cardioprotective GPCR, GPR37L1.

12:20-12:23 pm **Kailun Lee (Garvan)**. XBP1 is required for β-cell compensation during metabolic stress.

12:23-12:26 pm **Katherine Tonks (Garvan)**. Individualised multidisciplinary management of gestational diabetes with protocolised frequent follow-up results in fewer neonatal special care nursery admissions in private practice.

Lunch: Poster Session ***12:30-2:10 pm - Poster Marking 12.50-2.00 pm***
(Please remove all posters BEFORE afternoon session)

Session 3 2:10-3:10 pm

2:10-3:10 pm **Rising Stars (3×20 min) Chair: A/Prof Alex Viardot**

2:10-2:30 pm **Sophie Stocker (AMR).** *Optimising the use of vancomycin – using therapeutic drug monitoring to achieve precision medicine.*

2:30-2:50 pm **Ira Deveson (Garvan).** *Chiral DNA sequences as reference standards for clinical genomics.*

2:50-3:10 pm **Charles Cox (VCCRI).** *Using the force: Piezo channels as molecular reporters of mechanical forces.*

Afternoon Break 3:10 - 3:30 pm

3:30-4:00 pm **PLENARY. Chair: Dr Chris Stanley**

Prof Gemma Figtree (University of Sydney, Northern Clinical School, Kolling Institute of Medical Research, Charles Perkins Centre)

4:00-4:45 pm **Panel Discussion on 'Healthy Ageing'. Chair: A/Prof Roger Chen**

Panel members: **A/Prof Andrew Jabbour, Prof Geraint Rogers, Dr Joanne Reed, Dr Andy Philp**

4:45-5:00 pm **Closing Remarks and Prizes**

5:00-7:00 pm Cocktail Function

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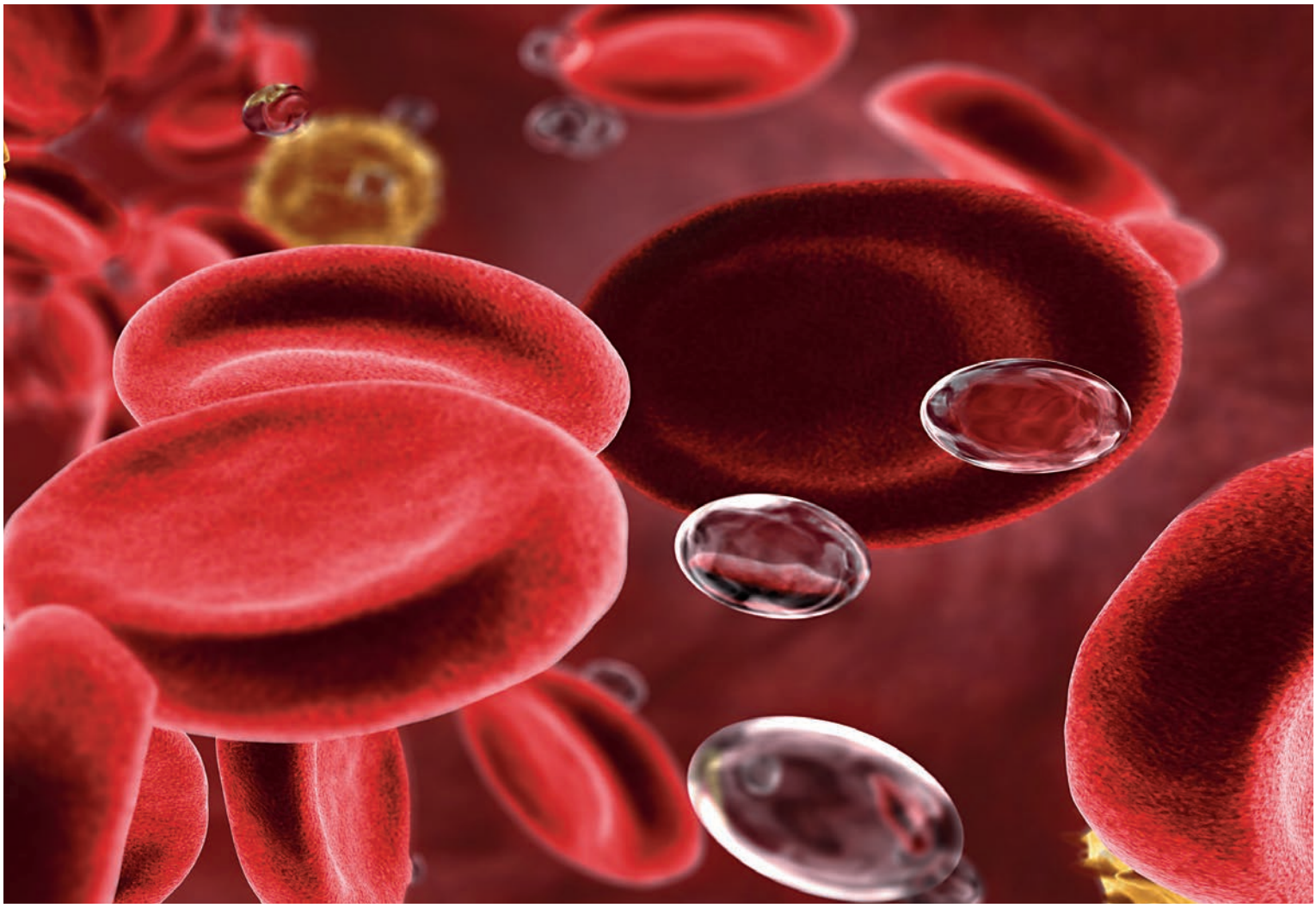

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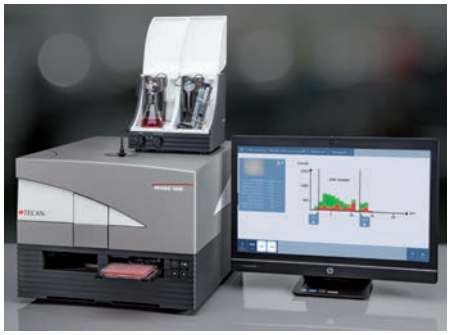

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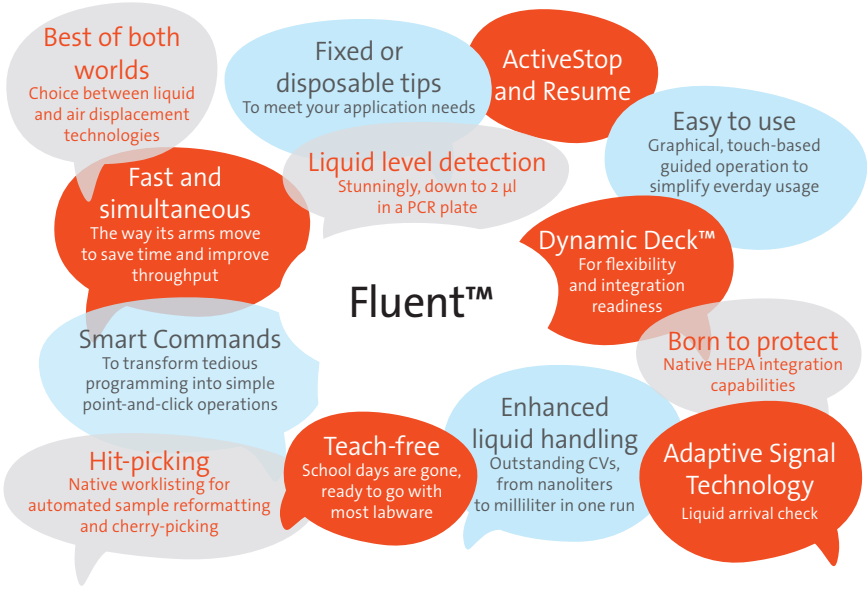

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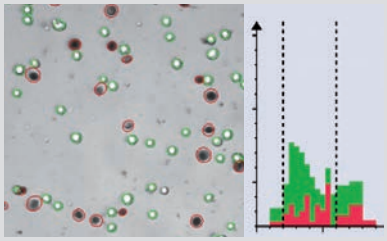

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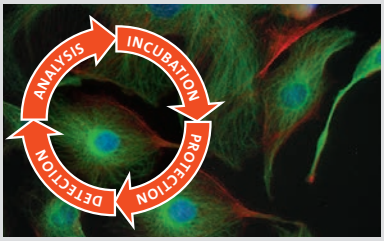

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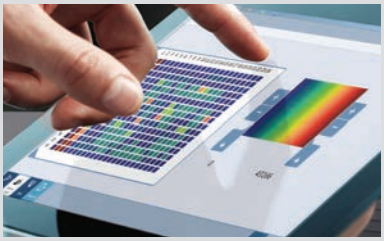

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7th Annual St Vincent's Campus PostDoc Symposium Abstracts
13th September 2018

Session 1: Oral Talks

Novel circulating biomarkers identify insulin resistance phenotypes in obesity

Yen Chin Koay[1,2], PengYi Yang[3], Daniel L Chen[4], Arthur B Jenkins[4,5], Jerry R Greenfield[4,6,7], John F. O'Sullivan[1,2,8*], Dorit Samocha-Bonet[4,5*]

[1]Sydney Medical School, The University of Sydney, Australia [2]Heart Research Institute, Sydney, Australia [3]School of Mathematics and Statistics, University of Sydney, Australia [4]Diabetes and Metabolism Division, Garvan Institute of Medical Research, Sydney, Australia [5]School of Medicine, University of Wollongong [6]St Vincent's Clinical School, Faculty of Medicine, UNSW, Sydney, Australia [7]Department of Endocrinology and Diabetes Centre, St Vincent's Hospital, Sydney, Australia [8]Royal Prince Alfred Hospital, Department of Cardiology, Sydney, Australia
**Equally-contributing authors*

9:15-9:30 am

Objective: Measurement of insulin resistance may ultimately assist in guiding the most effective therapy in type 2 diabetes (T2D). We aimed to identify circulating biomarkers of muscle and liver insulin resistance in obesity to guide treatment in the clinical setting.

Methods: Metabolomics and lipidomics profiling by LC/MS were combined with a specialized machine-learning algorithm to identify plasma biomarkers that characterize muscle and liver insulin resistance in a cohort of 62 individuals with obesity (BMI range 31-48 kg/m^2) phenotyped using the gold-standard 2-step hyperinsulinaemic-euglycaemic clamp with deuterated glucose to evaluate glucose regulation in muscle and liver.

Results: Fourteen circulating metabolites and lipids were closely correlated with muscle insulin resistance (Spearman *rho*>0.2, p<0.05), while nineteen were associated with hepatic insulin resistance (Spearman *rho*>0.3, p<0.05). A hybrid learning model that combines clustering-based prototype selection and random forest-based feature analysis identified two triacylglycerols (TAGs) and a phosphatidylcholine (PC) in plasma as the best classifiers differentiating between the liver and muscle insulin resistance phenotypes, followed by select metabolites, clinical features, and biochemical parameters. The three lipids identified by the hybrid learning model far out-performed standard clinical measures, including fasting plasma glucose, 2-h plasma glucose post 75 g oral glucose load and glycosylated haemoglobin (HbA1c), classifying 61 of 62 subjects correctly.

Conclusions: We provide a simple novel tool based on circulating lipids and metabolites to guide physicians to the most effective insulin-sensitising treatment in individuals with obesity. Future studies comparing the efficacy of the biomarker-guided therapy with the traditional treatment are necessary.

Cyclin E2 promotes, but cyclin E1 opposes, genome instability via rereplication in cancer cells

Christine S.L. Lee[1], Samuel Rogers[2], Niantao Deng[1], Kristine Fernandez[1], Sarah Alexandrou[1], C. Marcelo Sergio[1], Abhijit Kulkarni[3], Lucy Gugasyan[3], Elizabeth A. Musgrove[4], Andrew Deans[5], Andrew Burgess[6,7], C. Elizabeth Caldon[1,8]

[1]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW, Australia 2010 [2]Children's Medical Research Institute, 214 Hawkesbury Road, Westmead NSW 2145, Australia [3]Monash Pathology, Level 4, Monash Medical Centre 246 Clayton Road, Clayton, Victoria 3168 [4]Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Glasgow, United Kingdom G61 1BD [5]St Vincent's Institute, 9 Princes Street Fitzroy Victoria 3065 Australia [6]ANZAC Research Institute, Concord, New South Wales 2139, Australia [7]The University of Sydney Concord Clinical School, Faculty of Medicine and Health Concord, New South Wales 2139, Australia [8]St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, NSW Australia 2052

9:30-9:45 am

Background: Cyclin E1 and cyclin E2 are pro-proliferative proteins whose dysregulation in cancer promotes genomic instability. Cyclin E1 causes genomic instability through under-replication of DNA and faulty cell divisions, but cyclin E2 does not engage the same pathways. We hypothesised that cyclin E2 induces genomic instability by subverting its normal developmental role in genome rereplication to form polyploid cells.

Methods: We established models of whole genome rereplication in cancer cells, and examined whether cyclin E1 and E2 influence rereplication to alter cellular ploidy and genomic stability.

Results: Cyclin E2, unlike cyclin E1, increased rereplication and polyploidy, leading to increased chromosome rearrangements and chromosome number. In re-replicating cells, cyclin E2 localises on chromatin to the prereplication complexes (preRC) that enable the initiation of new rounds of DNA replication. We establish that cyclin E2 promotes preRC formation by recruiting the preRC protein MCM7. In contrast, cyclin E1 upregulation decreases the availability of soluble preRC components and decreases loading of Cdt1, the preRC licensing factor. We show that cells protect themselves from rereplication damage by degrading cyclin E2. Finally, an examination of public datasets identifies that cyclin E2 is frequently amplified and that it correlates with genomic instability across different cancer subtypes.

Discussion: Despite cyclins E1 and E2 being regarded as interchangeable proteins, we have identified that cyclin E2 uniquely influences cancer genome evolution by increasing DNA rereplication. This is highly significant as cyclin E2 is amplified in ~10% of cancers and could potentially be targeted with CDK inhibitors.

PLENARY: Dr Andrew Philp

"Live Strong and Prosper – the role of skeletal muscle function in healthy ageing"

9:45-10:30 am

Abstract: Ageing is associated with a progressive decline in skeletal muscle mass and strength, a condition known as sarcopenia. Although variable across individuals, it has been estimated that loss of skeletal muscle occurs at a rate of 1-2% per year after the age of 50, with losses in strength occurring more rapidly, such that older muscles become disproportionately weak. Sarcopenia leads to increased frailty, loss of mobility, an increased risk of falls/fractures, a diminished quality of life, and in some cases, premature mortality. 3.7 million people in Australia are currently over 65 years (one-in-seven), with projections suggesting this number will more than double to ~8.7 million by 2046 (one-in-five). Whilst the health relevance of sarcopenia is recognised, the mechanistic cause of this condition is currently unknown. Work in the past decade has identified that elderly individuals have a blunted protein synthetic response to anabolic stimuli such as amino acids and resistance exercise. This inability of the elderly to optimally respond to anabolic stimuli has been termed anabolic resistance, and is proposed to be central in the progression of sarcopenia. The aim of this session will be to highlight current understanding of anabolic resistance in the context of sarcopenia and discuss exercise and nutritional approaches to counteract sarcopenia and promote healthy ageing.

Bio: Dr Andy Philp is a group leader in the Diabetes and Metabolism Division at the Garvan Institute of Medical Research, where he leads the Mitochondrial Metabolism and Ageing laboratory. Andy completed his PhD in Exercise Physiology at the University of Brighton UK, before completing post-doctoral training at the University of Dundee and the University of California Davis. Andy set up his independent research group at the University of Birmingham UK in 2012 where he joined as a Lecturer in Integrative Physiology, progressing to Senior Lecturer in 2015 before joining Garvan as a Group Leader in January 2018.

Andy's current research is focused on understanding how physiological stimuli such as exercise; inactivity and nutrition induce molecular signaling networks to remodel skeletal muscle in the context of health and disease. His group at the Garvan explores the role of mitochondrial metabolism in the progression of muscle deterioration in Diabetes and Ageing, focusing on the therapeutic potential of exercise, pharmacology and nutraceuticals to maintain optimal muscle function across healthspan. To achieve these goals, Andy's group utilizes cell, worm, rodent and human experimental models in combination with 'omic' platforms to provide detailed metabolic characterization of skeletal muscle.

Session 2: Flash Talks

Dissecting molecular causation of hypoplastic left heart syndrome using induced pluripotent stem cells

Hananeh Fonoudi[1,2], Alexis Bosman[1], David Humphreys[1], Ralph Patrick[1], Gillian Blue[3], Adam Hill[1], Joshua Ho[1], David Winlaw[3], Richard Harvey[1,2]

[1]Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia, [2]St. Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia, [3]The Heart Centre for Children, The Children's Hospital at Westmead, Sydney, NSW Australia

11:07-11:14 am

Hypoplastic left heart (HLH) is a genetically complex disease, characterized by underdevelopment of the left side of the heart. Despite being one of the most severe forms of congenital heart disease, little is known about its molecular and genetic underpinnings. Here, we have generated an *in vitro* model of HLH using human induced pluripotent stem cells (hiPSCs) to uncover pathogenic factors. hiPSCs were generated from 10 HLH patients and their parents (trio design; 3 clones per individual; 87 hiPSC lines in total). To investigate early cardiovascular development, hiPSCs were differentiated using embryoid-body and cardiac-directed differentiation methods, and their cellular populations and transcriptome were studied. Gene expression analysis of spontaneously differentiated cells showed lower expression of cardiac and vascular smooth muscle markers in patients compared to controls. Flow cytometry analysis performed after directed cardiac differentiation at 5-day intervals (day 0-30) showed perturbation of cardiomyocyte differentiation in HLH-hiPSCs. Time-course mRNA sequencing of 5 HLH families revealed that the highest differences between patients and parents were at day 20 post-differentiation initiation, with down-regulation of cell cycle being the main driver. This was further confirmed using another 5 independent HLH families. Cell phenotyping and functional analysis based on calcium flux properties also indicated HLH-cardiomyocytes were more immature. In summary, our findings suggest that the progression of cardiogenesis and vasculogenesis in HLH-hiPSCs is perturbed, which may include defects in the proliferation and maturation of the cells. Our data suggest a common pathogenic pathway underlying the formation of HLH, despite the genetic heterogeneity of disease causation.

NAD deficiency induced by gene-environment interactions as a cause of congenital malformation

Hartmut Cuny[1]

[1]*Victor Chang Cardiac Research Institute, Darlinghurst, Australia*

11:14-11:21 am

Congenital malformations and miscarriages are common and represent a major health problem. Many malformations require surgical correction and affected individuals often need medical support throughout their life. Causes for congenital malformations and miscarriages can be genetic, environmental, or gene-environment interactions, but in most cases the specific cause is unknown. We previously showed that homozygous loss-of-function mutations in two genes required for synthesis of NAD from tryptophan, *HAAO* and *KYNU*, result in miscarriage and multiple malformations in humans and mice, and defects could be prevented by supplementation with NAD precursors during pregnancy. We hypothesised that other genetic and/or environmental factors causing NAD deficiency also lead to miscarriages and birth defects.

Here, we show that pregnant mice without genetic mutation have similarly affected offspring when exposed to diets restricted in tryptophan and NAD precursors throughout their pregnancy. The observed embryo defects due to this environmental factor are the same types as those caused by homozygous *Haao* mutations. Furthermore, mothers with heterozygous *Haao* mutations and fed with the same NAD precursor-restricted diets have a higher prevalence of embryo defects, indicating a gene-environment interaction. Enzymatic measurement of NAD levels in embryos and their mothers' liver confirm that reduced NAD in the mother and embryo correlates with defects.

Our findings show that maternal NAD deficiency, due to genetic mutations, malnutrition or a combination of both, is a risk factor for adverse pregnancy outcomes. Screening NAD levels of pregnant women and correcting deficits via nutritional supplementation could potentially prevent a proportion of miscarriages and birth defects.

Singlet molecular oxygen regulates vascular tone and blood pressure in Inflammation

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[†]These authors contributed equally to this manuscript

11:21-11:28 am

Background: Singlet molecular oxygen ($^1O_2$) has well-established roles in photosynthetic plants, bacteria and fungi, but not in mammals. $^1O_2$ oxidizes amino acids including tryptophan to precursors of *N*-formylkynurenine. Enzymatic oxidation of tryptophan to *N*-formylkynurenine is catalyzed by a family of dioxygenases, including indoleamine 2,3-dioxygenase 1 (Ido1). Under inflammatory conditions, this heme-containing enzyme becomes expressed in arterial endothelial cells, where it contributes to the regulation of blood pressure. We hypothesise that Ido1 is capable of forming $^1O_2$ and this is the mechanism by which Ido1 contributes to blood pressure control in inflammatory conditions.

Methods and Results: Using analytical biochemistry, enzymology and physiology we show that arterial Ido1 regulates blood pressure via formation of $^1O_2$. We observed that in the presence of hydrogen peroxide, the enzyme generates $^1O_2$ and that this is associated with the stereo-selective oxidation of tryptophan to a tricyclic hydroperoxide via a previously unrecognized oxidative dioxygenase activity. The tryptophan-derived hydroperoxide acts as a signaling molecule, inducing arterial relaxation and decreasing blood pressure in mice, in a manner dependent on cysteine residue 42 of protein kinase G1α. Our findings demonstrate a pathophysiological role for $^1O_2$ in mammals via formation of an amino acid-derived hydroperoxide that regulates vascular tone and blood pressure during inflammatory conditions.

Conclusion: Here we show that Ido1 inhibition is a potential therapeutic target for treatment of hypotension associated with severe inflammation. In the broader context, we expect that our findings will be the starting point for more sophisticated redox signaling by hydrogen peroxide and expand the biological roles of Ido1. For example, other heme-containing enzymes known to produce $^1O_2$ could participate in such redox signaling.

Investigating population variability using high throughput electrophysiological phenotyping of human induced pluripotent stem cell-derived cardiomyocytes

Melissa M. Mangala[1], Matthew Perry[1,2], Jamie Vandenberg[1,2] and Adam Hill[1,2].

[1]Victor Chang Cardiac Research Institute, [2]St. Vincent's Clinical School, UNSW

11:28-11:35 am

The recent genomics revolution hopes to deliver an era of precision medicine. However, our ability to link primary genotypes to clinical phenotype is still limited. For example, Long QT syndrome (LQTS) type 2 is caused by mutations in *KCNH2*, which encodes the hERG protein that carries the rapid delayed rectifier K^+ current (I_{Kr}) – a major component of cardiac repolarisation. Loss of function mutations in *KCNH2* result in prolongation of the cardiac action potential and hence the QT interval on the surface electrocardiogram and increase risk of fatal arrhythmias. However, even in patients with the same primary mutation, phenotype can be highly variable. Similarly, in the acquired form of LQTS, which occurs as a result of drug block of hERG, proarrhythmic risk is highly variable across the population. One of the major factors that is thought to contribute to this phenomenon is the varied genetic background between individuals which alters the electrical context in which the primary insult must be considered. In this study we have used a panel of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) combined with high throughput phenotyping to assess the role of variable genetic background in determining phenotype. RNA transcript levels of forty key ion channel genes, measured using Nanostring assays, varied between 1.1 and 22-fold across our cell lines. Electrophysiology and calcium handling phenotypes were measured in the same cell lines using kinetic imaging cytometry highlighting significant differences in the observed phenotypes between populations. For example, for two normal cell lines, the action potential duration measured at 75 % repolarisation (APD_{75}) was 440 +/-50 ms (SD; n=5400 cells) and 317 +/- 81 ms (SD; n = 800 cells). These results demonstrate that an approach incorporating genetically diverse iPSC lines and high throughout phenotyping provides a basis for quantitative analysis of the role of genetic variability in phenotypic presentation of arrhythmic cardiac electrical disorders. The principals learned will apply to the implementation of precision medicine to other inherited heart rhythm problems, and at a broader level all genetically determined diseases.

Intravital optical window imaging of RhoA-, Rac1- and Akt-FRET biosensor mice monitoring drug treatment response in cancer.

Nobis M.[1], Warren, S.C.[1], Herrmann, D.[1], Conway, J.R.W.[1], Melenec, P.[1], Stoehr J.[1], McCulloch, A.T.[1], Floerchinger A.[1], Murphy K.J.[1], Welch, H.C.E.[3], Haigh, J.J.[4], Sansom, O.J.[2], Morton, J.P.[2], Strathdee, D.[2], Blyth, K.[2], Pajic, M.[1], Anderson, K.I.[5], Timpson, P.[1]

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11:35-11:42 am

Background: Small GTPases such as Rac1 and RhoA enable cells to migrate during development as well as metastasize during cancer progression by actively remodelling the cytoskeleton of cells. Co-option of this activity has been demonstrated both in mammary and pancreatic cancer. Furthermore, in pancreatic cancer the PI3K pathway is aberrantly regulated in ~21% of cases.

Methods: More specific, time-resolved monitoring of these key drivers ranging from an *in vitro* to *in vivo* settings can be achieved by the use of FRET-biosensors and genetically engineered mice expressing these biosensors to track protein activity and the effect of therapeutic intervention. This was achieved using time-correlated single photon counting (TCSPC) fluorescent lifetime imaging (FLIM) on a multiphoton system in conjunction with optical windows.

Results: Here, we describe the generation and characterization of these FRET-biosensor mice to examine RhoA, Rac1 and Akt kinase activity in an *in vivo* setting in a variety of cell types in homeostasis as well as in mouse models of cancer. Elevated levels of Rac1 and RhoA activity were observed in models of invasive mammary and pancreatic cancer such as the polyoma-middle-T-antigen (PyMT) model and the KPC ($KRas^{G12D/+}$ and $p53^{R172H/+}$ driven) model. There, spatially defined to the invasive borders, high small GTPase activity was observed, absent in non-invasive mouse models such as the KC ($KRas^{G12D/+}$ alone) and KPflC ($KRas^{G12D/+}$ and p53 KO). Finally, spatiotemporally resolved imaging of the inhibition of RhoA, Rac1 and Akt activity live *in vivo* was achieved by employing optical windows implanted on top of developed tumours. Treatment was monitored for a period of up to 24h and the therapeutic response.

Discussion: This imaging allowed for unprecedented insight into treatment dynamics and the strong potential for further tailoring of targeted therapeutics in *in vivo* settings. Using these FRET biosensor mice represents a strong resource in understanding tissue context specific signalling events during cancer progression and drug target validation *in vivo*.

Whole Genome Sequencing Reveals Elevated Tumor Mutational Burden and Initiating Driver Mutations in African Men with Treatment-Naive High-Risk Prostate Cancer

Weerachai Jaratlerdsiri[1]

[1]*Garvan Institute of Medical Research, Darlinghurst, Australia*

11:42-11:49 am

African American men are more likely to die from prostate cancer than any other racial group. The contribution of acquired genomic variation to this racial disparity is largely unknown, while genomic data is lacking for Africa. Here we performed the first tumor-normal paired deep whole-genome sequencing for Africa. Providing a direct study-matched comparison between African- and European-derived treatment-naïve high-risk prostate tumors for 15 cases, allowed for further comparative analyses of existing data. Excluding for a single hyper-mutated tumor (55 mutations per megabase), we observed a 1.8-fold increase in small somatic variants in African versus European-derived tumors (t-test, P-value=1.02e-04), rising to 4-fold when compared with published tumor-matched data. Furthermore, we observed an increase in oncogenic driver mutations in African tumors (t-test, P-value=2.92e-03); roughly 30% impacted genes novel to prostate cancer, while 79% of recurrent driver mutations appeared early in tumorigenesis. Conversely, complex genomic rearrangements were less frequent in our African tumors, although we describe a uniquely hyper-duplicated tumor (149 transposable elements). Comparable to African Americans, ERG fusions and PIK3CA mutations were absent, and PTEN loss less frequent. CCND1 and MYC were frequently gained, with somatic copy number changes more likely to occur late in tumorigenesis. In addition to traditional prostate cancer gene pathways, genes regulating Calcium ion-ATPase signal transduction were notably disrupted in our African tumors. Although preliminary, our results call for further validation and investigation into the potential implication for elevated tumor mutational burden and tumor-initiating mutations in clinically unfavorable prostate cancer to improve patient outcomes in Africa.

Inhibition of Vascular Smooth Muscle Cell Migration by Enzymatically-Active, Truncated Heme Oxygenase-1

Kong SMY, Ni J, Newington D, Dunn LL, Ayer A, Suarna C, Lam M, Maghzal G, and Stocker R

Vascular Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst NSW 2010, Australia

11:49-11:56 am

Heme oxygenase-1 (Hmox1) is an ~32 kDa enzyme that degrades heme to CO, Fe^{2+} and biliverdin, and that plays a primary role in iron homeostasis. Previous studies utilising a pharmacological approach showed that chemical inducers of Hmox1 attenuate cardiovascular disease, including angioplasty- and stent-induced neointimal hyperplasia and vascular disease. We observed that treatment of rat aortic vascular smooth muscle cells (RASMCs) with stressors such as hypoxia or hemin increased the formation of a truncated form of Hmox1. Mass spectrometric analysis confirmed truncated Hmox1 to lack 23 C-terminal amino acids ($Hmox1_{\Delta 23}$). Fractionation of hypoxia-treated RASMCs indicated $Hmox1_{\Delta 23}$ expression to be partly present in the nucleus. This was also evidenced by confocal microscopy using FLAG-Hmox1-6HIS constructs. Nuclei isolated from hypoxia-treated RASMCs had significantly increased heme oxygenase activity, as assessed by the conversion of heme to biliverdin, determined by liquid chromatography-mass spectrometry. Purified $Hmox1_{\Delta 23}$ was also shown to retain enzymatic activity. Over-expression of $Hmox1_{\Delta 23}$ in RASMCs significantly decreased cell migration, and this was attenuated by mutation of both the His25 required for heme binding and a putative nuclear localisation signal of Hmox1. We also identified an amino acid sequence within the C-terminus for stress-induced 'truncation' of Hmox1. Adenoviral delivery of different Hmox1 isoforms and mutants found over-expression of $Hmox1_{\Delta 23}$ significantly inhibited neointimal hyperplasia *in vivo* using a rat carotid balloon injury model. Taken together, this data demonstrates that enzymatically-active, truncated Hmox1 inhibits vascular smooth muscle migration and has potential as a therapeutic to reduce angioplasty- and stent-induced neointimal hyperplasia and vascular disease.

Session 3: Oral Talks

A potential novel pathway to regulate cellular concentrations of the essential lipid coenzyme Q

Anita Ayer[1,5], Ghassan J. Maghzal[1], Jelske N. van der Veen[2], Ian W. Dawes[3], Dennis E. Vance[2], Catherine F. Clarke[4], René L. Jacobs[2] and Roland Stocker[1,5]

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1:30-1:45 pm

Background: Coenzyme Q (CoQ) is an essential lipid, and CoQ deficiency is implicated in diseases *e.g.* heart failure and diabetes. Currently, CoQ supplements are the only strategy available to overcome CoQ deficiencies, but the low bioavailability of supplemental CoQ is a significant limitation. Targeting steps regulating CoQ content may be a novel strategy to enrich cellular CoQ content in disease.
Methods: We performed a genome-wide screen of ~5,000 yeast mutants and measured CoQ content in each mutant using HPLC-EC to identify genes critical for CoQ homeostasis.
Results: ~ 30 mutants were identified with significantly higher CoQ content compared to WT, including the *cho2* mutant. *CHO2* encodes a phosphatidylethanolamine (PE) methyltransferase that catalyses the conversion of PE to phosphatidylcholine. *cho2* mutants contained five times more CoQ than WT, and displayed a significantly increased rate of CoQ synthesis. Homologs of *CHO2* are found in mammals (*PEMT)* indicating a potential conserved role for this gene in CoQ regulation. To investigate if $Pemt^{-/-}$ mice also display elevated CoQ content akin to yeast *CHO2* mutants, we analysed CoQ content in $Pemt^{+/+}$ and $Pemt^{-/-}$ mice. CoQ content was ~ double in $Pemt^{-/-}$ compared to $Pemt^{+/+}$ in the liver and adipose tissue from animals fed high-fat diet. This phenotype was reversed by liver-specific expression of PEMT indicating a direct relationship between *Pemt* expression and CoQ content.
Discussion: Our data suggests a novel role for PEMT in CoQ content regulation and modulating PEMT activity may be a novel approach to enrich tissue CoQ content.

Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque

Imran Rashid[1*], Ghassan J. Maghzal[1,2*], Yung-Chih Chen[3*], David Cheng[1], Jihan Talib[1], Darren Newington[1], Minqin Ren[4], Saumitra K. Vajandar[4], Amy Searle[3], Ana Maluenda[3], Eva-Lotte Lindstedt[5], Andrew Jabbour[1,6], Antony J. Kettle[7], Andre Bongers[8], Carl Power[8], Erik Michaëlsson[5], Karlheinz Peter[3], and Roland Stocker[1,2]

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**These authors contributed equally*

1:45-2:00 pm

Background: Myeloperoxidase (MPO) is an inflammatory enzyme that is abundantly expressed in high-risk culprit (unstable) coronary plaques responsible for fatal acute myocardial infarction. We hypothesised that MPO may be a valid target for intervention and possess diagnostic value. Thus, we investigated the role of MPO in the development of unstable atherosclerotic plaques.

Methods: The tandem stenosis model of plaque instability was employed in apolipoprotein E gene knockout (*Apoe*$^{-/-}$) mice. We tested the role of MPO using both *Mpo*$^{-/-}$*Apoe*$^{-/-}$ and the pharmacological inhibition of MPO activity using a 2-thioxanthine. We determined MPO activity by detection of 2-chloroethidium using liquid chromatography-tandem mass spectrometry and *bis*-5HT-DTPA-Gd (MPO-Gd)-enhanced magnetic resonance imaging (MRI). Plaque phenotype was histologically verified. Data were analysed for normality and significance was determined using the appropriate parametric or non-parametric tests.

Results: MPO activity was two-fold greater in unstable compared to stable plaque. Genetic deletion of *Mpo* or pharmacological inhibition of MPO activity increased fibrous cap thickness and decreased fibrin and hemosiderin content in unstable plaque. Lesion monocytes, red blood cell markers, circulating leukocytes and lipids remained unaffected by treatment. MPO-Gd MRI demonstrated sustained enhancement of unstable plaque on T1-weighted imaging that was two-fold greater than stable plaque, which was significantly attenuated by deletion or pharmacological inhibition of MPO.

Discussion: Our data suggests a role for MPO in atherosclerotic plaque instability and that non-invasive imaging and pharmacological inhibition of MPO activity has immense clinical translational potential for the management of high-risk coronary artery disease.

Unravelling the fate of cardiac PDGFRA$^+$ cells in healthy and injured mouse hearts using single cell RNA-SEQ

Dorison A[1], Farbehi N[1], Patrick R[1], Forte E[1], Du J[1], Janbandhu V[1], Harvey RP[1,2]

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2:00-2:15 pm

Myocardial infarction (MI) and subsequent heart failure are among the leading causes of mortality worldwide.

Previously, we have demonstrated that SCA1$^+$PDGFRα$^+$ (S+P+) cardiac stromal cells contain a colony-forming fraction similar to mesenchymal stem cells in bone marrow. We hypothesise that cardiac PDGFRα$^+$ cells encompass progenitor cells involved in cardiac homeostasis and repair after injury.

To unravel the differentiation potential of S+P+ cells, we permanently tagged PDGFRα$^+$ cells. Labelled cells isolated from the ventricles of sham-operated and MI mice 7 days post-surgery were then analysed using single-cell RNA-seq. Each main cell cluster has been validated by flow cytometry and mapped by immunohistochemistry.

The transcriptomes of more than 14000 cells have been explored, allowing us to identify 9 and 11 clusters in Sham and MI, respectively, including several sub-populations, as within fibroblasts (FB), which give rise to myofibroblasts after MI. Moreover, we could identify immune cells deriving mainly from the myeloid lineage. While differentiation of FB into endothelial cells (ECs) is controversial, we have found a significant EC population in Sham and MI hearts as well as a mural cell cluster. These results suggested that PDGFRα-derived cells participated to neo-vascularization post-injury. Finally, a distinct cycling cell cluster demonstrated the proliferative nature of the lineage tagged cells.

Our study provides an in-depth analysis of the cardiac stromal compartment in homeostasis and disease, and provide us with a comprehensive view of the fate of PDGFRα$^+$ cells after injury. This approach may lead to the identification of new therapeutic strategies to improve cardiac repair.

Cognitive decline is associated with an accelerated rate of bone loss and increased fracture risk in elderly population

Dana Bliuc[1]

[1]*Garvan Institute of Medical Research, Darlinghurst, Australia*

2:15-2:30 pm

Dementia and osteoporosis are common among elderly with some studies suggesting a causal link. Longitudinal studies that assess the complex relationships among cognitive decline, bone loss and fracture risk independent of ageing are lacking.

We aimed to determine the association between: 1) cognitive decline and bone loss, and 2) significant cognitive decline (≥3 points) on Mini Mental State Examination (MMSE) (baseline (Y0) - Year 5 (Y5)) and fracture risk (Y5 - 15).

A cohort of 3287 women and men 65+ from population-based Canadian Multicentre Osteoporosis Study (CaM*os)* was followed for 15 years. Association between bone loss and cognitive decline was estimated using mixed-effects models, and fracture risk using Cox Proportional Hazards models. Models were adjusted for age, education and co-morbidities.

Over 95% of participants had normal cognition at baseline. Annual % change in MMSE was similar for women [-0.33 (IQR:-1.00 to +0.02)] and men [-0.33 (IQR:-0.72 to 0.00)]. After accounting for confounders, cognitive decline was significantly associated with bone loss (0.13%/year MMSE loss for each 1%/year BMD loss). Approximately 13% of participants experienced significant cognitive decline by Y5. In this group, fracture risk was increased only in women [HR, 1.45 (95% CI: 1.00 to 2.10)]. After adjustment for bone loss the magnitude decreased slightly [HR, 1.39 (95% CI, 0.93-2.06)].

This study showed a significant association between cognitive decline and bone loss. Women with significant cognitive decline experienced increased fracture risk, which was only marginally explained by bone loss. Further studies are needed to determine mechanisms that link these common conditions.

Identification of clinically actionable variants from genome sequencing of families with CHD

Dimuthu Alankarage, Eddie Ip, Justin O. Szot, Jacob Munro, Gillian M. Blue, Katrina Harrison, Hartmut Cuny, Annabelle Enriquez, Michael Troup, David T. Humphreys, Meredith Wilson, Richard P. Harvey, Gary F. Sholler, Robert M. Graham, Joshua W. K. Ho, Edwin P. Kirk, Nicholas Pachter, Gavin Chapman, David S. Winlaw, Eleni Giannoulatou, Sally L. Dunwoodie

Victor Chang Cardiac Research Institute, Darlinghurst, Australia

2:30-2:45 pm

Background: Congenital heart disease (CHD) affects up to 1% of live births. However, a genetic diagnosis is not made in most cases. The purpose of this study was to assess the outcomes of genome sequencing (GS) of a heterogeneous cohort of CHD patients.

Methods: 97 families, with probands born with CHD requiring surgical correction, were recruited for genome sequencing. At minimum, a proband-parents trio was sequenced per family. GS data were analyzed via a two-tiered method: application of a high-confidence gene screen (hcCHD), and comprehensive analysis. Identified variants were assessed for pathogenicity using the ACMG-AMP guidelines.

Results: Clinically relevant genetic variants in known and emerging CHD genes were identified. The hcCHD screen identified a clinically actionable variant in 22% of families. Subsequent comprehensive analysis identified a clinically actionable variant in an additional 9% of families in genes with recent disease associations. Overall, this two-tiered approach provided a clinically relevant variant for 31% of families.

Discussion: Interrogating GS data using our two-tiered method allowed identification of variants with high clinical utility in a third of our heterogeneous cohort. However, association of emerging genes with CHD etiology, and development of novel technologies for variant assessment and interpretation will increase diagnostic yield during future reassessment of our GS data.

Session 4: Oral Talks

DNA barcoding demonstrates immuno-editing of metastatic breast cancer cells at the clonal level

Simon Junankar[1,2], Jessica Yang[1], Chia-Ling Chan[1], Breanna Fitzpatrick[1], Andrea McFarland[1], Alex Swarbrick[1,2]

[1]Garvan Institute of Medical Research, Darlinghurst, NSW 2010, [2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Darlinghurst, NSW 2010

3:15-3:30 pm

The majority of cancer patients die of metastatic disease. Currently, immunotherapy is one of the few successful therapeutic modalities for treating metastatic disease. Unfortunately many cancers exhibit resistance to currently approved immunotherapies. We hypothesise that innate resistance of cancer cells can explain some of this failure to respond to immunotherapy.

To test whether clonal selection of innately resistant cells can drive resistance to immunotherapy, we used cellular DNA barcoding, a powerful technique that allows for the analysis of cancer cell dynamics over time at the clonal level. We introduced a DNA barcode library (ClonTracer) into murine metastatic breast cancer cells (4T1) so that each cell receives a single unique barcode. These barcodes can then be "read" using next-generation sequencing. Following orthotopic transplantation with these barcoded cells I resect the primary tumour and allow metastases to develop. I have compared the barcodes detected at metastatic sites of wild-type mice with immuno-compromised mice; in addition I have compared wild-type mice treated with the combination anti-PD1/anti-CTLA4 immunotherapy with control treated mice.

These studies have demonstrated that the immune system reduces the number of 4T1 clones in the metastatic lung, and the number of clones is further reduced following immunotherapy. In addition I have detected a specific subset of highly metastatic clones in immunocompromised mice that are preferentially eliminated following immunotherapy. We now aim to identify what makes these clones more susceptible to immunotherapy using RNAseq analysis. We hope this will identify pathways that can be targeted to improve immunotherapy response in cancer patients.

Decoding the identity and flux of cardiac cells in injury and homeostasis at single-cell resolution

Ralph Patrick[1,2,4], Nona Farbehi[1,4,5], Aude Dorison[1,4], Munira Xaymardan[3], Vaibhao Janbandhu[1,2,4], Joshua W. K. Ho[1,2], Robert Nordon[5] and Richard P. Harvey[1,2,4]

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3:30-3:45 pm

Ischaemic heart disease, including myocardial infarction (MI), is the leading cause of death in Australia for both men and woman. After MI, a billion cardiomyocytes may die and will not be replaced. However, insights into cardiac repair mechanisms in mammals and lower animals has spurred interest in developing heart regeneration strategies for man. Cardiac injury triggers a complex cascade of cellular and molecular events that control the injury response. Understanding cardiac sub-lineages and their response to injury, including the communication and signaling networks that control the injury response, will be critical for developing strategies to augment cardiac repair and achieve heart regeneration in man.

In this study we have implemented single-cell RNA-seq (scRNA-seq) on over 30,000 cardiac cells investigating the impact of MI on adult mouse hearts. Through unbiased clustering analysis of single-cell transcriptomes we have profiled the response of major cardiac cell lineages and previously unstudied cell sub-populations to injury. Diffusion Map analysis of short-term lineage-traced fibroblasts revealed the differentiation trajectories from immature fibroblast populations to myofibroblasts. Through cell-cell communication analysis with ligand-receptor networks we further define a putative communication network of cardiac cell sub-types across injury-response and homeostasis. Together, these data provide an unparalleled, high-resolution map of the cellular heterogeneity of the interstitial and stromal compartments of the heart and their responses to injury.

PLENARY: Dr Joanne Reed

"Age-associated B Cells: The ABC's of Autoimmune Disease"

3:45-4:45 pm

Abstract: Sjögren's syndrome is one of the most prevalent autoimmune diseases and affects women between 40-60 years of age. The development and persistence of B cells that have "gone rogue" and produce autoantibodies is central to disease pathogenesis. The aim of this research was to determine why rogue B cells arise in previously healthy adults. Mass spectrometry sequencing of purified serum autoantibodies was paired with massively parallel sequencing of peripheral blood B cells to identify rogue B cells expressing the serum autoantibody. Using the unique autoantibody sequence as a barcode, single cell RNAseq and targeted genomic DNA sequencing was used to compare rogue B cells to polyclonal B cells from the same patient. Rogue B cells expressed an aberrant gene expression profile consistent with previously described age-associated B cells. Longitudinal evaluation of a patient with Sjögren's syndrome revealed a rogue B cell clone that persisted over 6 years. The clone acquired mutations in the autoantibody itself and in a gene previously reported to be mutated in multiple cases of leukemia and lymphoma. The acquisition of these mutations coincided with the development of severe autoimmune pathology. These data support a model where rogue B cells acquire mutations that corrupt their gene expression and internal regulatory circuits and suggest a common root to the pathogenesis of autoimmunity and B cell malignancy.

Bio: Dr Joanne Reed is a Group Leader in the Immunology Division at the Garvan Institute. She completed her PhD at Flinders University in Adelaide. She then received an NHMRC CJ Martin Fellowship for postdoctoral training at New York University and Australian National University. Since moving to Garvan, Joanne has developed single cell genomic approaches to study B cells responsible for severe autoimmune pathology in patients with autoimmune disease. Her work aims to identify targeted therapies for autoimmune disease.

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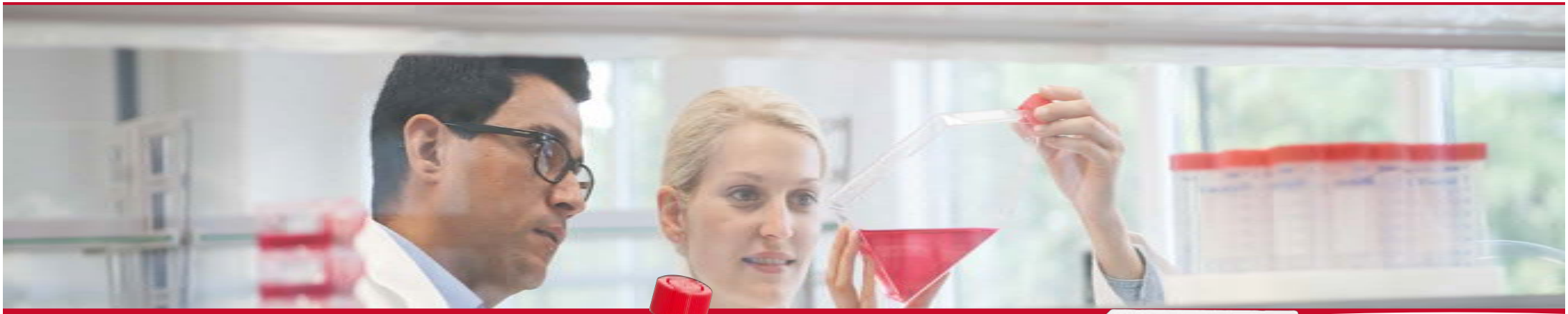

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depth/depθ/ *n*. **1** sagacity; wisdom. **2** the range of one's understanding or competence. **3** complete detail; thoroughness.

expertise/ɛkspəːˈtiːz/ *n*.**1** special skill, knowledge, or judgment in a particular area; expertness; know-how. **2** the condition of being an expert.

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26th Annual St Vincent's Campus Research Symposium Abstracts
14th September 2018

Session 1: Oral Talks

An innovative HIV testing initiative: the NSW dried blood spot self-sampling HIV testing pilot program

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9:00-9:10 am

Background: The NSW Dried Blood Spot (DBS) Self-Sampling HIV Testing Pilot Program was developed as part of the NSW HIV Strategy 2020 as an innovative initiative to target the estimated 11% of individuals living with undiagnosed HIV infection. Commencing in 2016, phase one of this project initially offered an at-home capillary sample collection kit whereby eligible participants ordered a DBS kit online and sent their self-collected sample via Australia Post to St Vincent's Hospital Centre for Applied Medical Research (AMR), Sydney for an HIV-1/2 antibody screen. From September 2016 the project evolved to include hepatitis C RNA testing for participants who identify as either Aboriginal or Torres Strait Islander (ATSI) or a person who injects drugs (PWID). The second phase of this project consists of DBS kits being sent to approved sites to either distribute or assist participants in sample collection and the website registration process. Based on site-specific applications (SSA), results are managed either by the sites clinician's or by the Sexual Health InfoLink (SHIL) based at Sydney Sexual Health Centre, Sydney hospital.

Objectives: To provide an easily accessible pathway to HIV and hepatitis C screening for at-risk populations who have barriers to testing via conventional methods.

Methods: To determine eligibility, participants answer survey questions (https://www.hivtest.health.gov.au) which in turn generates a 5-digit validation code that is transcribed onto the DBS card linking the person registering, to the person providing the sample. Having either ordered the kit online via the website or collected the kit at an approved site, the DBS kit contains two sets of ancillary items to ensure that five 1cm drops of blood are collected for the DBS testing algorithm to be completed. Once the DBS card is received in the lab it is assessed for sample adequacy and an HIV antibody ELISA is completed followed by hepatitis C RNA testing if requested. Reactive HIV antibody results are confirmed by a western blot and HIV-1 nucleic acid test. For any reactive results, the patient is contacted directly and linked based on residential postcode to the closest clinic for conventional venepuncture confirmatory serology. Participants are provided counselling and support via the staff at SHIL throughout this process. An automatic message is generated for negative results and sent to the patients preferred method of contact, SMS or email.

Results: As of the 31st of July 2018, there have been 1158 registrations with 753 DBS cards returned and tested for HIV (65% return rate) and 109 tested for hepatitis C RNA. 6 patients have tested reactive for HIV with 15 patients receiving a reactive hepatitis C RNA result and been linked into care. 1317 DBS kits have been distributed throughout approved sites within NSW with additional sites being recruited.

Conclusions: The DBS pilot project has demonstrated to be an innovative and successful model, providing a free and confidential service allowing at-risk populations convenient access to HIV and hepatitis C testing and linkage to care. The project will continue to be funded for a further two years keeping in-line with the NSW HIV Strategy 2020.

Disclosure of Interest Statement:
NSW Health funds St Vincent's Hospital Reference Laboratory and SHIL to run the DBS pilot. NSW Health does not receive funding from pharmaceutical companies for HIV or hepatitis C therapies.

Retrospective review of 64 patients with amiodarone-induced thyrotoxicosis

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9:10-9:20 am

Background: Amiodarone-induced thyrotoxicosis (AIT) can cause cardiac decompensation. Type 1 (T1) is treated with anti-thyroid medications (ATM) and Type 2 (T2) with glucocorticoids (GC). Differentiating between types is challenging. This study aimed to evaluate the management of AIT at St Vincent's Hospital.

Methods: Retrospective audit of 64 patients treated for AIT (2007-2016). T1 or T2 classification was based on radiological criteria.

Results: Mean age was 60±2y; 81% were male. Initial treatment was ATM in 23, GC in 17 and combination (COMB) in 24. Treatment groups had similar age, gender, cardiac comorbidities and fT3. Median fT4 was 28pmol/L (19-33) in ATM, 40pmol/L (29-47) in GC and 55pmol/L (39-75) in COMB (p=0.002). Proportion of T1 and T2 did not differ between treatment groups. Initial therapy induced euthyroidism in 52% of patients (70% in ATM, 53% GC and 33% COMB; p=0.045). Response rate to ATM was the same when only T1 were considered whereas response to GC was higher (83%) when only T2 were included. A further 11% required the addition of a second medication. Thyroidectomy was undertaken in 33%. Compared to patients who responded to medication, thyroidectomy patients were younger (54±3 vs 63±2y; p=0.03) and had higher prevalence of cardiac failure (81% vs 53%; p=0.09). Despite median American Society of Anesthesiologists classification 4, no patient experienced cardiorespiratory complications/death.

Discussion: Patients with AIT had poor response to initial treatment. The poorest response was observed in COMB group, likely related to more severe hyperthyroidism. Thyroidectomy is safe if performed with expertise in cardiac anaesthesia.

Gastric emptying rate and the safety of GLP-1 receptor agonists in Prader-Willi syndrome

Amanda Hor[1,2,3], Renee Richens[1], Saesha D'Silva[3], Jarron Dodds[1], Georgina Loughnan[4], Tania Markovic[4], Lesley Campbell[1,2,3], Alexander Viardot[1,2,3].

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9:20-9:30 am

Introduction: One of the most challenging behaviours in Prader-Willi Syndrome (PWS) is the insatiable appetite with uncontrolled hyperphagia, for which no effective pharmacologic treatment exists, and this often leads to obesity and its complications. GLP-1 agonists have been used in treating type 2 diabetes mellitus in PWS and non-PWS individuals. GLP-1 decreases food intake by increasing satiety centrally and slowing gastric emptying (GE). As delayed GE has been reported in PWS, further slowing is undesirable due to a theoretical increased risk of gastric necrosis. Therefore, it is important to determine the effect of GLP-1 agonists on gastric motility in PWS before recommending its use.

Objectives: To determine the gastrointestinal safety of extended-release exenatide in PWS by assessing its effects on GE.

Methods: This is an ongoing prospective interventional study where subjects with PWS with normal gastric emptying are treated with once-weekly exenatide for 12 weeks. Gastric emptying was assessed by gastric scintigraphy at baseline, 4 weeks and 12 weeks. Gastric scintigraphy involves eating a 99mTc-labelled breakfast (486kCal) and images are acquired immediately after the meal and 1, 2 and 4 hours thereafter. Blood sample collection and appetite assessments were performed at regular intervals along with a cognitive function assessment. For safety measures, standard gastric emptying rates in healthy people were established using both lean and overweight/obese control subjects. Gastric emptying rate of PWS subjects were assessed at baseline; those whose 4-hour gastric retentions fall above the 95th percentile of the standards established by the controls will not enter the treatment arm. A second gastric emptying assessment is performed after 4 weeks of treatment. Treatment is discontinued if gastric retentions fall above the 95th percentile of the control standards.
A third gastric emptying assessment is performed after 12 weeks for those who continue with treatment beyond 4 weeks.

Results: 26 subjects in total, 9 lean, 7 overweight/obese and 10 PWS aged between 18-51 were recruited. 5 male and 5 female PWS subjects (mean age 25 years, 19-39) were recruited. All PWS subjects had a genetic diagnosis of Prader –Willi syndrome. 10 PWS subjects completed the baseline gastric scintigraphy assessment while 7 completed the 4-week assessment and 4 completed the 12-week assessment. Baseline gastric retention at 4 hours for lean and overweight/obese controls were 5.8% and 9.6% respectively while PWS had a higher gastric retention of 12.7%. 8 PWS subjects entered the treatment arm while 2 were excluded due to high baseline gastric retention at 4 hours (43.9% and 30.1%). In the treatment arm, 7 PWS subjects had a mean gastric retention of 12.2% at 4 weeks while 4 PWS subjects had a mean of 11.4% at 12 weeks respectively, compared to 6.7% at baseline. 1 PWS subject's extended-release exenatide treatment was ceased at 4 weeks due to high gastric retention of 19.9%. This increased from 8% at baseline. In the treatment arm, mean weight was 95.7kg at baseline; 93.4kg and 101.4kg at 4 weeks and 12 weeks respectively.

Conclusion: GLP-1 agonists appear to delay gastric emptying in PWS after 4 weeks of treatment. This persisted until the final 12-week assessment. We suggest using GLP-1 agonists cautiously in PWS until their gastric motility effects are fully established, to avoid increasing the risk of gastric rupture and necrosis.

Mechanisms underpinning resistance to CDK4/6 inhibition in ER+ breast cancer

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9:30-9:40 am

Background: Endocrine resistant estrogen receptor positive (ER+) breast cancers are particularly dependent upon cyclin-dependent kinases (CDK) 4/6 for proliferation. As such, potent CDK4/6 inhibitors (CDK4/6i) have been integrated into clinical practise for treatment of advanced ER+ breast cancers. Despite initial efficacy, acquired resistance to CDK4/6i is already emerging by mechanisms which remain unknown. Our aim is to develop clinically relevant in vitro and in vivo models of CDK4/6i and endocrine therapy to identify the mechanisms of acquired resistance in breast cancer.

Methods: Using MCF-7 breast cancer cells we have generated a palbociclib resistant (PalbR) cell line. To complement this, we are developing a panel of in vitro and in vivo models that mimic the clinical treatment of patients. Here, palbociclib is combined with an endocrine therapy; tamoxifen, fulvestrant, or long-term estrogen deprivation to mimic aromatase inhibition. Furthermore, we have developed a patient-derived xenograft model resistant to chronic fulvestrant and palbociclib treatment.

Results: We have identified several potential mechanisms of palbociclib resistance whereby increased proliferation was induced by an increase in estrogen-response genes and a reduction of the CDK inhibitor proteins p21 and p27. Mechanistically, loss of p27 de-represses CDK2 activity, and we show that PalbR and MCF-7 cells resistant to combined palbociclib and tamoxifen therapy have enhanced sensitivity to the CDK2 inhibitor CYC065.

Conclusions: Our novel panel of acquired resistance models demonstrate differences in cellular morphology and growth trajectories, and our analysis has identified mechanisms of CDK4/6i resistance and provides insight into novel therapeutic combinations.

Lack of protein prenylation promotes the assembly of NLRP3-dependent inflammasomes in a cell culture model of mevalonate kinase deficiency (MKD)

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9:40-9:50 am

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Mevalonate kinase deficiency (MKD) is an autoinflammatory disease caused by mutations in an enzyme of the mevalonate pathway, leading to loss of isoprenoid lipids necessary for protein prenylation. It has been proposed recently that defective prenylation in macrophages leads to activation of the pyrin inflammasome and IL-1 release, contradicting previous evidence supporting NLRP3 inflammasome involvement. To address this controversy, we used a cell culture model in which prenylation in human THP-1 monocytes was inhibited by treatment with simvastatin (SIM).

SIM pre-treatment significantly increased the proportion of cells with an ASC-containing inflammasome speck, increased caspase-1 activity and caused a 3-fold increase in the release of IL-1 and IL-18 after stimulation with LPS, a TLR4 agonist. SIM had a similar effect after stimulation with the TLR2 agonist Pam3CSK4. These effects of SIM were completely abolished by the addition of geranylgeraniol, an isoprenoid lipid that restored prenylation in SIM-treated cells. After CRISPR/Cas9 editing, lack of NLRP3 prevented the formation of inflammasome specks, caspase-1 activation and IL-1 release in SIM-pre-treated cells stimulated with LPS, but lack of pyrin had no effect. In addition, MCC950 (a specific inhibitor of NLRP3 inflammasome formation) completely prevented caspase-1 activation, IL-1 release and pyroptosis in SIM-pretreated cells stimulated with LPS. Importantly, MCC950 also completely abolished the excessive IL-1 release from LPS-stimulated PBMCs from an MKD patient.

Our observations demonstrate that lack of prenylation in human monocytes, a model of MKD, leads to enhanced formation of NLRP3-dependent, not pyrin-dependent, inflammasomes after stimulation with TLR2/4 agonists.

PLENARY: Prof Geraint Rogers

"The influence of the human microbiome on healthy ageing"

9:50-10:20 am

Abstract: The human microbiome is integral to many aspects of human physiology, including the regulation of metabolism and innate and adaptive immunity. A growing body of research suggests that changes in the composition and function of the microbiome that are associated with ageing can contribute to reduced bone density, increased frailty, risk of dementia, and susceptibility to infection. This presentation will provide an overview of the mechanisms by which the microbiome influences the ageing process, and the potential for novel strategies that target the microbiome to facilitate healthy ageing will be discussed.

Bio: Geraint Rogers is Director of Microbiome Research at the South Australian Health and Medical Research Institute, leads a research group at the Flinders University School of Medicine, and is a Matthew Flinders Research Fellow. His research focuses on understanding how the human microbiome influences health and disease.

Session 2: Oral Talks

Post-Fracture Mortality: A latent class analysis of multimorbidities

Thao P. Ho-Le[1], Thach Tran[2], Jacqueline R. Center[2,3], John A. Eisman[2,3,4], Tuan V. Nguyen[1,2,3,4,5].

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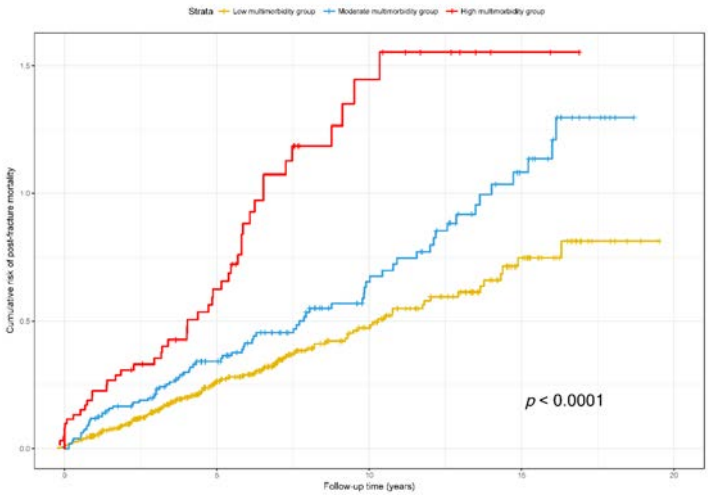
11:00-11:10 am

Osteoporosis frequently coexists with other chronic diseases (i.e. multimorbidity), but the relationship between multimorbidity (≥ 2 diseases) and mortality is unclear. We sought to define the pattern of multimorbidity and its impact on the risk of post-fracture mortality.

This study involved 890 women and 244 men having fracture. During over 20-year follow-up, health status of participants had been recorded. Osteoarthritis, cardiovascular disease, type II diabetes, cancer, rheumatoid arthritis, neurological illnesses, and mental illnesses were ascertained at baseline. We used latent class analysis (LCA) to define the pattern of diseases co-occurrence within an individual. The relationship between the LCA-derived clusters of diseases and post-fracture mortality was assessed by the Cox's proportional hazard model.

The prevalence of multimorbidity was 38% in women and 35% in men. Multimorbidity was associated with increased risk of post-fracture mortality (HR 2.4, 95%CI:1.68-3.38). The LCA identified 3 clusters of patients: low (68%), moderate (20%), and high (12%) multimorbidity groups. The 5-year risk of post-fracture mortality among the high multimorbidity group was 45%, which was 1.64-fold higher than the moderate multimorbidity group, and 2.3-fold higher than the low multimorbidity group. The increased risk of death was mainly seen among individuals with cardiovascular disease (HR 1.6, 95%CI: 1.2-2.3), type II diabetes (HR 1.8, 95%CI:1.0-3.2), rheumatoid arthritis (HR 1.8, 95%CI: 1.1-2.9) and neurological illnesses (HR 2.74, 95%CI: 1.2-6.3). The proportion of post-fracture mortality attributable to multimorbidity was ~30%.

These findings suggest that ~ one-third of individuals with a fracture have multimorbidity, and that the coexistence of morbidities accounts for one-third of post-fracture mortality. These emphasize the need for a wholistic management of patients having fracture.



Visceral fat and insulin resistance is associated with lower bone turnover

Angela Sheu[1]

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11:10-11:20 am

Background: Despite higher bone mineral density (BMD) in obesity and type 2 diabetes (T2DM), fracture risk may be increased, possibly due to reduced bone quality. Not all individuals with obesity develop T2DM, and insulin resistance (IR) is associated with increasing visceral adipose tissue (VAT). The relative effects of obesity, VAT and IR on bone health remain unclear. We aimed to assess the relationship between bone turnover markers (BTM), BMD and body composition in insulin-sensitive lean (IS-L), insulin-sensitive overweight (IS-O), insulin resistant (IR) and T2DM subjects.

Methods: Concurrent whole body scans and fasting plasma samples in 525 subjects from the Dubbo Osteoporosis Epidemiology Study were analysed for BMD, body composition, VAT, IR (Homeostasis Model Assessment [HOMA] ≥2.5) and BTM (osteocalcin [OC], procollagen type 1 N-propeptide [P1NP] and C-terminal telopeptide [CTx]). Predictive modelling was performed using Bayesian Model Averaging.

Results: BMD was lower in IS-L compared with IS-O, IR and T2DM (no differences within the obese groups). VAT increased progressively from IS-L to IS-O, IR and T2DM (0.6±0.4, 1.2±0.6, 1.5±0.8, 1.8±0.9kg, $p<0.0001$, respectively). BTM were lower only in T2DM. VAT independently predicted bone formation (-6.6%/kg VAT for P1NP, $p=0.02$; -9.3%/kg VAT for OC, $p<0.0001$) while HOMA independently predicted bone resorption (-0.11%/% change in HOMA for CTX, $p=0.005$).

Discussion: Obesity was associated with higher BMD, but BTM were lower only in T2DM. VAT correlated with bone formation while IR correlated with bone resorption. Thus, VAT and IR, not total body fat, is associated with lower bone turnover and may contribute to fracture risk in T2DM.

Gaining 'MUMentum': two randomised controlled trials evaluating brief internet-delivered cognitive behavioural therapy for perinatal anxiety and depression

Siobhan Loughnan[1]

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11:20-11:30 am

Background: Perinatal anxiety and depression is common and associated with significant adverse outcomes; yet access to evidence-based treatment is limited. Internet-delivered CBT (iCBT) has demonstrated effectiveness in treating postpartum depression symptoms, yet no published studies have investigated iCBT for generalized anxiety symptoms, with or without depression. In two randomised controlled trials, we evaluated the efficacy of two, 3-lesson interventions – MUMentum Pregnancy and MUMentum Postnatal – in reducing anxiety and depression symptoms in pregnant and postpartum women.

Methods: For both RCTs, we recruited 218 women, between 13-30 weeks gestation/within 12mths postpartum, who met clinical threshold on self-report measures of depression (PHQ-9)[7] and/or anxiety (GAD-7)[8] and were randomized to iCBT or treatment as usual (TAU) control group. Outcomes were: self-reported anxiety, depression, psychological distress, maternal attachment, parenting confidence, and quality of life (QOL). Measures were assessed at pre-, post-treatment and at four-week follow-up.

Results: Both trials demonstrated high rates of participant adherence (75%), satisfaction. Overall, both MUMentum programs resulted in significant reductions in primary outcomes from pre- to post-treatment and significant improvements in secondary outcomes. Specifically, intent-to-treat mixed model analyses indicated MUMentum Postnatal resulted in moderate to large between-group superiority over TAU at post-treatment for anxiety (Hedges' g=0.78), depression (g=0.99), distress (g=1.69), attachment (g=0.70), and QOL (g>0.47), with gains maintained at follow-up.

Conclusions: This study demonstrates preliminary efficacy and acceptability of brief, unguided iCBT as a treatment option for pregnant and postpartum women experiencing clinical levels of anxiety and depression; and has significant implications for increasing treatment accessibility.

Landscape of the breast cancer tumour microenvironment using single-cell RNA sequencing

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11:30-11:40 am

Breast cancers are a complex 'ecosystem' of diverse cell types, whose heterotypic interactions between malignant, stromal and immune populations are central in defining the aetiology of the disease and response to therapy. Despite advances in other carcinomas, including immune-checkpoint blockade in melanoma, combinational therapies that target the supporting microenvironment have made little clinical progress in breast cancer treatment. The development and implementation of such therapies has been largely impeded by a poor understanding of the cellular heterogeneity within breast cancers, which is masked using conventional bulk sequencing approaches.

Single-cell RNA sequencing (scRNA-Seq) technologies and computational methods have emerged as remarkable tools for studying the diverse cellular populations within the tumour microenvironment. Using this approach, we comprehensively profiled more than 100,000 neoplastic, immune and parenchymal cells sampled from 22 primary and metastatic breast cancers collected at surgery and biopsies. At single-cell resolution, we comprehensively describe novel immune and stromal subsets and infer their intra-tumour interactions, leading to important mechanistic insights with therapeutic implications. In addition, we profiled the heterogeneity of cancer-associated fibroblasts (CAFs) across primary and metastatic sites and propose multi-faceted roles in regulating malignancy and tumour immunology. Novel cell surface markers identified using scRNA-Seq allow us to prospectively isolate CAF subsets for the validation of targetable gene expression features.

This is by far the largest and most comprehensive single cell genomic study in any cancer to date. Our approach highlights the power of single cell technologies to unravel the complexities of the tumour microenvironment and identify novel mechanisms underlying carcinogenesis. Such insights will guide the next-generation of therapies, which will likely be based upon an integrated understanding of the neoplastic, stromal and immune states that define a tumour and inform treatment response in breast cancer.

The acute glucose lowering effect of IC7Fc is dependent upon functional pancreatic secretion

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11:40-11:50 am

Background: gp130 receptor ligands are now recognised as potential therapeutical strategies for the treatment of type 2 diabetes (T2D). However, cytokines have previously failed in clinical trials. Accordingly, we engineered a novel chimera, termed IC7Fc, and studied its acute effect on glucose homeostasis.

Methods: Diet-induced obese C57BL/6J mice (4-week HFD) received a single IC7Fc (1 mg/kg i.p.) or saline injection and glycaemia was monitored. We next metabolically examined aged diabetic db/db mice upon short-term treatment. In another model we studied mice that received the β cell toxin Streptozotocin (STZ; 55 mg kg-1) to induce partial pancreatic damage. To characterise the whole-body effect on glucose homeostasis we conducted IC7Fc-Euglycaemic clamps in the presence of the pancreatic inhibitor Octreotide (7.5 µg kg-1 min-1).

Results: In diet-induced obese mice a single dose of IC7Fc reduced blood glucose within 90 min and increased plasma insulin and C-peptide. IC7Fc reduced blood glucose in young db/db mice within 90 min. The actions progressively diminished along with age. IC7Fc was ineffective at reducing blood glucose in STZ-treated mice. Clamp experiments show that the glucose infusion rate needed to maintain euglycaemia was greater with IC7Fc compared with saline (8.45 ± 1.23 mg kg-1 min-1; $P<0.05$). Additionally, IC7Fc inhibits hepatic glucose output and stimulates glucose disposal, which was abolished in the presence of Octreotide.

Conclusions: It is clear that the acute effects of IC7Fc are largely dependent upon functional β cells. However, the IC7Fc remains an attractive novel treatment for obesity and the progression of T2D in humans.

Diabetes outreach clinic for the homeless: experience and outcomes over a 3 years period

Alexander Viardot[1,2,3]

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11:50-11:53 am

Introduction: St Vincent's Hospital Sydney (SVH) is located in the inner city serving people with poverty and homelessness. While disadvantaged groups are at higher risk of developing diabetes, they often fail to access optimum care. The SVH Diabetes Service tested a Diabetes Outreach Clinic (DOC), utilizing a physician in the practice model, at a homeless men's hostel in 2012/13. After its initial success, the DOC was implemented permanently, and a 3-year experience is reported.

Methods: The Matthew Talbot Hostel Clinic (MTHC) is a nurse-run health facility located within the Hostel for Homeless Men. The SVH DOC comprises of a visiting Specialist Team (Endocrinologist and Diabetes Educator) and the primary care team (General Practitioner and Clinical Nurse Specialist for Homeless Health). Pre-selected patients with diabetes or metabolic syndrome were clinically assessed and screened for diabetes complications. The DOC aims were: to provide access to specialist diabetes care for homeless men, to up-skill local primary care teams, and to promote linkages between primary and tertiary care.

Results: Twenty DOCs were held in the MTHC over 36 months. 47 men were assessed in a total of 205 multidisciplinary case conferences. 21 patients were seen once. The remaining 26 patients were seen 7 times over 18 months (1-36 months) in average. All patients had adverse lifestyle and cardiovascular risk factors, and had difficulty accessing mainstream health services. The majority had underlying mental health problems. Despite this, most suggested treatment changes were followed, resulting in significant improvements in HbA1c, blood pressure and lipid profile. A key factor to success was the close supervision of treatment by the local primary care team.

Conclusions: This DOC offers an innovative and cost-effective model of diabetes care for vulnerable populations, improving treatment outcomes and supporting and up-skilling the primary care team.

Personalised medicine approach in pancreatic cancer reveals fine-tuned stromal FAK manipulation improves global response to gemcitabine and Abraxane while sensitising circulating tumour cells to shear stress in transit

Kendelle J. Murphy[1], Morghan C. Lucas[1], Claire Vennin[1], James R.W. Conway[1], Sean C. Warren[1], Joanna N. Skhinas[1], Romain Bidanel[1], Astrid Mangneau[1], Thomas R. Cox[1,2], Lisa Horvath[1], Yingxiao Wang[2], Jennifer P. Morton[4], Owen Sansom[4], Marina Pajic[1,2], David Herrmann[1,2*] and Paul Timpson[1,2*].

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11:53-11:56 am

Background: Pancreatic ductal adenocarcinoma (PDAC) is predicted to be the second leading cause of cancer mortality by 2030. PDAC development occurs in a complex microenvironment, where extensive stromal desmoplasia alters mechanical tumour-stromal interactions, promoting tumour progression and metastatic spread. Consequently, we aim to fine-tune manipulation both the tumour and stromal compartments in primary, transient and secondary sites, while improving global response to standard-of-care, gemcitabine/Abraxane.

Methods: Intravital imaging of the FUCCI cell cycle reporter, in parallel with Second Harmonic Generation (SHG) imaging of collagen fibres, was used to dynamically monitor tumour cell response to standard-of-care therapy, gemcitabine/Abraxane and extra cellular matrix (ECM) organisation respectively. To complement our in vivo metastatic studies, we used both primary PDAC cell lines and patient-derived xenograft cell lines in 2D and 3D in vitro models of invasion, anchorage-independent growth and shear-stress assays

Results: Intravital imaging of the FUCCI cell cycle reporter and parallel SHG at primary sites revealed that fine-tuned FAKi decrease ECM remodelling and reduces cell cycle progression. Further imaging of secondary sites, post intrasplenic injection, was used to systematically demonstrated that FAKi also sensitising cells to shear stress prior to standard-of-care therapy, enhancing treatment efficacy whilst reducing metastatic spread.

Furthermore, stratified patient samples suggest a subset of patients with high FAK activity are likely to respond to FAK priming regimes.

Discussion: This fine-tuned stromal manipulation may allow us to maximise gemcitabine/Abraxane therapy whilst reducing drug toxicity and potentially reducing further metastatic spread in patients.

Fractures and fracture-associated mortality attributable to low bone mineral density and advancing age: a time-variant analysis

Ha Mai[1]

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11:56-11:59 am

Background: Although bone mineral density (BMD) is causally related to fracture, the burden of fractures attributable to low BMD has not been investigated. In this study, we estimated the fraction of different fracture types occurring in older people that can be attributed to low BMD.

Methods: The study involved 2,320 women and 1,380 men aged 50 years and older, whose bone health has been continuously monitored for up to 20 years. During the follow-up period, the incidence of fractures was ascertained by X-ray report. Femoral neck BMD was measured at baseline by GE-LUNAR DXA, and expressed as T-scores. Osteoporosis was defined as T-scores being less than -2.5. Advancing age was categorized as age from 70 years old. The estimation of time-dependent attributable fraction (AF) was based on the Cox's proportional hazards model.

Results: Overall, 21% of women and 11% of men had osteoporosis by BMD. In univariable analysis, approximately 21% and 16% of total fractures in women and men, respectively, were attributable to osteoporosis. When osteoporosis was combined with advancing age, the two factors accounted for 46% and 51% of total fractures in women and men, respectively. However, the two factors (age and osteoporosis) accounted for ~80% of all hip fractures. Fracture was associated with increased risk of mortality, and approximately 63% and 53% of postfracture mortality in women and men, respectively, were attributable to advancing age, osteoporosis and fracture; however, most of the attributable proportion was accounted for by advancing age.

Conclusion: While 80% of hip fractures were attributable to advancing age and osteoporotic BMD, these factors contributed to less than 50% of total fractures. Most of postfracture mortality was attributable to advancing age.

CD39+ T regulatory cell reconstitution in Multiple Sclerosis patients undergoing autologous haematopoietic stem cell transplantation

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11:59-12:02 pm

Background: Autologous haematopoietic stem cell transplantation (AHSCT) may induce remission in autoimmune diseases like Multiple Sclerosis (MS) by regenerating immune-regulatory cells, including antigen-specific CD39+ T regulatory cells (Tregs). CD39+ Tregs are defective in MS patients and their role in AHSCT for MS is yet to be determined. Variable CD39 expression and Treg function has been attributed to polymorphism of the CD39 gene.

Methods: We aimed to determine whether AHSCT reconstitutes CD39+ Tregs ($CD4+CD25^{hi}CD127^{lo}$) by flow cytometry and investigate CD39 genetic polymorphism in MS patients (n=13). Statistical analysis was performed using Mann-Whitney U and Wilcoxon's test.

Results: Before transplantation, we found a significant difference in CD39 antigen density (median fluorescence intensity [MFI]) of CD39+ Tregs in MS patients with different CD39 genotypes (mean MFI: 2152 vs 3347; $p<0.05$). CD39 MFI expression on CD39+ Tregs remained stable after transplantation in each genotype group for up to 12 months. Overall, a significant increase was detected in the proportions of CD39+ Tregs in the blood of MS patients at 3 ($p<0.0001$), 6 ($p<0.001$) and 12 months ($p<0.01$) post-transplantation compared to baseline. However, when grouped by CD39 genotype, the proportions of CD39+ Tregs were only significantly increased post-AHSCT in the patient cohort with lower CD39 MFI expression (baseline vs 3 [$p<0.05$], 6 [$p<0.01$] and 12 months [$p<0.01$]).

Discussion: Our study suggests that CD39 genetic polymorphism has a role in delineating Treg reconstitution in MS patients undergoing AHSCT. Differences in Treg reconstitution between patients with different CD39 genotypes may reflect different clinical responses towards AHSCT.

Hospitalisations over a year follow-up in a cohort of adults living with HIV with sustained viral suppression in Australia

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on behalf of the PAART study investigators

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12:02-12:05 pm

Background: Patients successfully treated with antiretroviral therapy (ART) for HIV develop few AIDS-defining events, are successfully aging and living longer. This longitudinal analysis aimed to understand the reasons and risks for hospitalisation.

Methods: We recruited a national cohort of adults living with HIV on stable ART at 17 sites. A 90-item survey recorded demographics, physical health, life stressors, social supports, HIV disclosure, stigma/discrimination, healthcare access, treatment adherence, side effects, health/treatment perceptions, and financial/employment status. Neurocognitive, clinical and virological data were collected; including hospitalisations over 12 months. Baseline variables that were bivariately associated with hospitalisation ($p<0.05$) in the following year of follow-up were included in a Cox proportional hazards regression model.

Results: Of 522 adults, 94.5% were male, mean age 50.8 years, mean HIV duration 12 years, median ART duration 11.0 years (IQR 1.2-6.8), median duration HIV RNA <50 copies/mL 3.3 years (IQR 1.2-6.8). Over 12 months, 94 (18.0%) participants had 143 hospitalisations. Hospitalisations were for various non-AIDS reasons including serious non-AIDS events (SNAEs) (see Table). Twenty-eight baseline variables bivariately associated ($p<0.05$) with hospitalisation over the following 12 months. However, the only variable significant in multivariable Cox regression was having started ART to prevent HIV disease progression (adjusted hazards ratio 0.6 [95% confidence interval 0.4-0.9] $p=0.029$).

Conclusions: In this population of adults with suppressed HIV, hospitalisations were common over 12 months, mostly for procedures (minor/diagnostic) or SNAEs. Preventable reasons for hospitalisation included accidents, renal issues and infection. Hospitalisations were largely not predictable, the only significant variable being starting ART to prevent HIV progression, potentially a surrogate for early HIV.

Table Reasons for hospitalisation

Reason for hospitalisation	Episodes, n (%)
Procedure – minor/diagnostic	25 (17.5)
SNAE	14 (9.8)
Accident/assault	13 (9.1)
Renal (stone/calculus/tumour/UTI)	11 (7.7)
Neurological	11 (7.7)
Infection	11 (7.7)
Cardiac (non-SNAE)	8 (5.6)
Other (various)	50 (34.9)
Total	143 (100)

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Mapping the heterogeneity of CCR5+ CD4 T cells by high dimensional flow cytometry

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12:05-12:08 pm

Background: The subset of CD4 T cells that express the cell surface chemokine receptor, CCR5, are the most important target of HIV-1 infection. However, the functions, phenotypes and anatomical locations of CCR5+ CD4 T cells are poorly understood.

Methods: 20-parameter flow cytometry using the 5-laser BD Symphony has been undertaken to better define CCR5+ CD4 T cells in peripheral blood in healthy adults. CCR5 staining, in combination with 19 other fluorochrome-labelled monoclonal antibodies (mAb), was optimized for the Symphony. Ultrasound-guided lymph node fine needle biopsies of axillary nodes were performed on healthy adult volunteers prior to and following Fluvax.

Results: Lymphocytes were gated on forward and side scatter, and CD3+ CD4+ T cells were gated on CD45RO+ memory cells. CCR5+ memory CD4 T cells were then analysed for expression of Treg markers (CD25hiCD127lo), chemokine receptors (CXCR5, CXCR3, CCR4, CCR6), c-type lectins (CD62L, CD161), integrins (□4, □7), activation markers (CD38, HLA-DR), and differentiation markers (CD27, CD28, CD73). Altogether, >150 different functional and trafficking phenotypes of CCR5+ CD4 T cells were seen, including Tregs, non-Tregs, gut-homing, non-gut homing, skin-homing, Th1, Th17, activated, resting, cytotoxic and non-cytotoxic cells. Also, activated CCR5+ CD4 T cells were greatly expanded in draining axillary lymph nodes at day 5 following Fluvax.

Discussion: These results reveal for the first time the extreme heterogeneity of CCR5+ CD4 T cells in blood and in lymphoid tissue, with significant implications for rational approaches to prophylaxis for HIV-1 infection and for purging of the HIV-1 reservoir in those already infected.

Functional analysis of a novel cardiac specific lncRNA

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12:08-12:11 pm

Background: A significant large amount of the mammalian genome previously found to not code for proteins and considered "junk", was found to actually specify a dynamic network of regulatory RNAs, termed long noncoding RNAs (lncRNAs). Here, we identified NkxUS, a heart-associated lncRNA, which lies upstream of the cardiac transcriptional regulator NKX2- 5. We hypothesise that NkxUS either regulates Nkx2-5 itself or other genes important for heart function and/or development. The aim of the study is to fully characterise NkxUS in order to identify its function.

Results: Our studies show that NkxUS is a long, cardiac and nuclear enriched transcript. It is expressed in the heart throughout development and a similar heart specific transcript occurs in humans. We found a heart rateassociated GWAS SNP, located within human NkxUS. This SNP lies within and disrupts a conserved RNA structure. Mice lacking part of the conserved structure exhibited a higher resting heart rate with no differences in conductance, suggesting a possible dysfunction in the sinoatrial node (SAN), the pacemaker of the heart. Currently, we are performing calcium imaging combined with drug treatments and pacing on dissected mouse SANs to identify the basis of the phenotype.

Discussion: Altogether, we present data on a novel cardiac enriched lncRNA and show that by deleting only 300bp, including the SNP homolog and part of the conserved structure, of a 10kb transcript, we get a similar phenotype as in humans. In summary, this project provides a detailed characterisation of NkxUS to analyse how it might impact heart function and development.

Reliability of Calcaneal Quantitative Ultrasound: a prelude to use in acute Charcot neuropathic osteoarthropathy

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12:11-12:14 pm

Background: charcot neuropathic osteoarthropathy (CN) is a rare, potentially devastating complication of diabetes mellitus. Novel tools to aid early diagnosis and monitoring of acute CN are needed. Calcaneal Quantitative Ultrasound (QUS) can detect early asymmetry in bone parameters between an individual's affected and unaffected foot. Advantages of this device include its portability, affordability, ease of use, and non-reliance on ionising radiation and contrast agents.
We aimed to assess the reproducibility of calcaneal QUS measurements.
Methods: healthy ambulant volunteers were recruited by advertisement. Calcaneal QUS was performed three times per foot with repositioning between measurements. A second observer repeated this process. Intraclass Correlation Coefficients (ICC) with 95% confidence intervals (CI) were calculated based on absolute agreement with a two-way mixed-effects model. Intra-observer ICC was obtained from the three single measurements and inter-observer ICC from the observer means.
Results: twenty participants (10 male, 40 feet) were included, with mean age 48.9±12.1 years, mean body mass index 25.2±4.3 kg/m^2 and 90% right foot dominant. ICC was high for Stiffness Index (SI) (Observer 1 0.97, 95% CI [0.96-0.99]; Observer 2 0.96, 95% CI [0.94-0.98]; inter-observer 0.98, 95% CI [0.96-1.00]), Speed of Sound (SOS) (Observer 1 0.97, 95% CI [0.95-0.99]; Observer 2 0.97, 95% CI [0.93-0.98]; inter-observer 0.98, 95% CI [0.97-0.99]) and Broadband Ultrasound Attenuation (BUA) (Observer 1 0.86, 95% CI [0.78-0.92]; Observer 2 0.80, 95% CI [0.69-0.88]; inter-observer 0.88, 95% CI [0.79-0.94]).
Conclusions: intra-observer and inter-observer reliability of calcaneal QUS was high. Future studies will examine the utility of calcaneal QUS in acute CN.

Immune reconstitution following autologous haematopoietic stem cell transplantation for multiple sclerosis is driven by sustained thymic reactivation.

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12:14-12:17 pm

Background: Autologous haematopoietic stem cell transplantation (AHSCT) is a promising strategy for Multiple Sclerosis (MS) patients that do not respond to conventional treatments, but the mechanisms enabling clinical improvement of MS in transplant recipients are not fully understood. We hypothesised that AHSCT induces a regeneration of thymic function, resulting in the re-development of a functional, tolerant immune system. We aimed to study recent thymic emigrants (RTE's) longitudinally by surface and DNA markers in a cohort of MS patients post-AHSCT to correlate with treatment response.

Methods: Peripheral blood mononuclear cells (PBMNCs) were collected from patients enrolled in the Phase II trial at St Vincent's Hospital Sydney for AHSCT in MS. A multicolour flow cytometry panel to optimally identify RTE's was performed on 10 patient samples at 0, 6, 12, 24 and 36 month timepoints, to track changes in thymic output following AHSCT. DNA markers of thymic function - Sj:b TREC ratio was performed in the same cohort of patients to enhance the validity of observed changes. Statistical analysis was performed with GraphPad Prism.

Results: A sustained, significant increase in RTE's and Sj:b TREC was detected between pre-transplant and 24 month post-transplant specimens ($n=10$, $p = 0.024$). Although there were smaller number of patients who had reached the 36mth timepoint, a similar trend occurred. A correlation between RTE's and Sj:b TREC was observed ($r=0.70$, $p = 0.003$). Contrary to other publications in the field, TREC as a marker of thymic output did not appear to be lower when patients were analysed by age (<30 yrs vs. >30 yrs). Greater thymic output as determined by Sj:b TREC was observed at all timepoints in patients who had evidence of sustained disease remission as opposed to patients who experienced disease relapse.

Conclusions: We observed that sustained thymic reactivation occurs following AHSCT for MS in this cohort of patients and this may contribute a durable clinical response.

Cardiovascular health of young and aged mice lacking the cardioprotective GPCR, GPR37L1

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12:17-12:20 pm

Background: GPR37L1 is an orphan G protein-coupled receptor highly expressed in glia, that has a proposed role in blood pressure (BP) homeostasis. Absence of GPR37L1 causes elevated BP in female mice and predisposes male mice to heart failure following cardiovascular stress. The aims of this study are: 1) to define the contribution of sympathetic nervous system activity to blood pressure in GPR37L1$^{-/-}$ mice, and 2) to investigate how ablation of GPR37L1 affects cardiovascular health with age.

Methods: Blood pressure, heart rate and physical activity was measured by radiotelemetry in young mice (14 weeks) under basal conditions, and during behavioural (feeding, dirty cage swap, restraint) or pharmacological stressors. Additionally, wildtype and GPR37L1$^{-/-}$ mice were aged to 52 weeks, and cardiovascular health was assessed by BP measurement (micromanometry) and tissue morphometry. Results analysed by two-way ANOVA, with Holm-Sidak post-hoc test.

Results: Female GPR37L1$^{-/-}$ mice have attenuated BP response compared to wildtype in both restraint (24.6mmHg vs 38.5mmHg, $P<0.05$) and dirty cage (23.8mmHg vs 30.7mmHg, $P<0.05$) stressors. This is consistent with trending reduction of sympathetic contribution to BP in GPR37L1$^{-/-}$ vs wildtype, as determined by power spectral analysis.

At 1 year of age, GPR37L1$^{-/-}$ mice do not have significantly different arterial BP from wildtype mice, though knockouts do show marked cardiac hypertrophy (heart weight to tibia length ratio: females 7.6mg/mm vs 7.2mg/mm, $P<0.05$; males 10.6mg/mm vs 9.2mg/mm $P<0.05$).

Discussion: Presence of GPR37L1 contributes to basal cardiovascular homeostasis, and deficits in signalling of this receptor may result in long term pathological changes.

XBP1 is required for β-cell compensation during metabolic stress

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12:20-12:23 pm

Pancreatic β-cells hypersecrete insulin to maintain normoglycaemia under metabolic stress conditions in a process called β-cell compensation. The role of endoplasmic reticulum (ER) stress and its associated unfolded protein response (UPR) in β-cell compensation is not clear. The transcription factor XBP1 regulates the adaptive UPR, which acts to alleviate ER stress by increasing protein folding capacity. The aim of this study was to examine the role of XBP1 in β-cell compensation. We generated β-cell-specific XBP1 knockout (β-XBP1KO) mice by crossing $XBP1^{flox}$ and $Pdx1\text{-}Cre^{ER}$ mice. Control and β-XBP1KO mice were fed chow or high-fat diet (HFD) for 4 weeks. Control mice fed HFD displayed normal fasting blood glucose levels and mild glucose intolerance. In contrast, β-XBP1KO mice fed HFD displayed increased fasting blood glucose levels, severe glucose intolerance, reduced insulin secretion and content, loss of adaptive UPR and β-cell identity gene expression and upregulation of the pro-apoptotic gene, *Trib3*. To investigate mechanisms, control and β-XBP1KO islets were cultured *ex vivo* under metabolic stress conditions induced by chronic (72h) palmitate (0.4mM) and high glucose (25mM) treatment. Cell death was potentiated in β-XBP1KO islets in association with reduced antioxidant gene expression, suggesting a diminished response to oxidative stress. The potentiated cell death in β-XBP1KO islets was prevented by co-treatment with an antioxidant, N-acetyl-cysteine. In conclusion, these studies suggest that XBP1 is required for β-cell compensation by regulating the UPR, β-cell identity and the antioxidant response. XBP1 may protect against type 2 diabetes by promoting insulin secretion and β-cell survival during metabolic stress.

Individualised multidisciplinary management of gestational diabetes with protocolised frequent follow-up results in fewer neonatal special care nursery admissions in private practice.

Wendy Bryant[1], Chelsea McMahon[1], Monika Fazekas-Lavu[1], Katherine Tonks[1,2,3]

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12:23-12:26 pm

Background: Gestational diabetes (GDM) affects approximately 10% of the Australian population. Management of GDM through public hospitals alone is not practical due to limited resources. There is a paucity of data of private models of GDM care.

Aims: This is the first report of results of an evidence-based program in a private GDM clinic in Australia.

Methods: Retrospective review of de-identified data for all women with GDM, and their babies, admitted for confinement to a Sydney private hospital from 1st March 2015 to 31st Dec 2016. We compared women who underwent treatment with Sydney Endocrinology (SE) to those whose GDM was managed privately or publically elsewhere. The SE multidisciplinary GDM clinic was founded with funding from a Friends of the Mater grant.

Results: Of 4287 births 390 babies were born to mothers with a history of GDM. Of these, 177 were managed through SE. The SE protocol includes an initial one-on-one multidisciplinary review with an endocrinologist, dietitian and diabetes educator, then weekly email contact, and further visits as required. Where possible, there is direct liaison with the patients obstetrician and midwife. SE patients did not differ from non-SE patients in age (34.9 vs 34.6 years), BMI (24.0 vs 24.1 kg/m^2), or ethnicity (all p=NS). There was no increase in the odds of elective or emergency Caesarian section (p=0.49, chi squared test for trend, Newcombe-Wilson method). SE patients had in increased odds of insulin and/or metformin prescription (49% vs 35%, respectively, OR 1.8, 95% CI 1.2-2.7, p=0.005). The SE babies did not differ from non-SE babies in gestational age at birth (mean 38.3 vs 38.2 weeks, respectively), weight (3177 +/- 519g vs 3238+/-477g, respectively), length (mean 50.6 vs 50.8 cm, respectively) or head circumference (all p=NS). There was a lower rate of special care nursery (SCN) admission for SE vs non-SE patients (23% vs 32%, respectively, OR 0.62, 95% CI 0.40-0.97, p=0.04). Similarly there were lower rates of hypoglycaemia (defined as <=2.5mmol/L, 36% vs 48%, respectively, OR 0.61, 95%CI 0.41-0.92, p=0.02). There was no difference in the mean lowest glucose of those that suffered hypoglycaemia in each group (2.0 vs 2.0 mmol/L, p=NS), rates of jaundice (32% vs 29%, p=0.7), nor foetal abnormality (7.3% vs 7.0%, p=0.8).

Discussion: In the private health care setting, GDM patients managed through a individualised multidisciplinary system with protocolised frequent follow-up are less likely to require SCN admission. This is likely related to more frequent initiation of insulin therapy, and is not associated with increased rates of neonatal hypoglycaemia, jaundice or malformation. We also postulate this is related to close frequent follow-up of patients, and extensive dietetic coaching and input. Further studies in this area would be useful to confirm these findings.

Session 3: Rising Stars
Dr Sophie Stocker

"Optimising the use of vancomycin – using therapeutic drug monitoring to achieve precision medicine"

2:10-2:30 pm

Abstract: Vancomycin is an antibiotic used to treat infections caused by methicillin-resistant *Staphylococcus aureus*. Therapeutic drug monitoring (TDM) is advocated for vancomycin due to its narrow therapeutic index. Dosing guidelines suggest target trough concentrations of 15-20 mg/L or an $AUC_{0\text{-}24}/MIC \geq 400$ mg.h.L^{-1}

Aims. To assess vancomycin prescribing according to guidelines, evaluate the attainment of therapeutic targets and identify factors associated with nephrotoxicity.

Methods: Vancomycin dosing history, patient demographics, pathology and incidence of acute kidney injury (AKI) and associated risk factors were collected retrospectively over a 9-month period from patients receiving vancomycin (>48 hours). Loading and maintenance doses and collection of drug concentrations were evaluated against guidelines. A Bayesian dose prediction software (DoseMe®) was used to estimate vancomycin drug exposure. It was assumed that the MIC was 1 mg/L. Vancomycin-associated AKI was defined according to van Hal et al. (2013).

Results: Plasma vancomycin concentrations (n=1043) were collected from 163 patients during 179 courses of therapy. The first dose prescribed was concordant with the guideline defined loading dose in only 24% of courses, while 72% were lower than guideline recommendations. In 42% of courses the initial maintenance dose was guideline concordant, while 24% exceeded the guidelines. Further, only 14% of blood samples were trough samples collected after the appropriate dose. Approximately 30% of the predicted trough concentrations were within 15-20 mg/L. Regardless, an $AUC_{0\text{-}24}/MIC \geq 400$ h was achieved at least once in 83% of courses. Patients who received doses (loading and maintenance) concordant with guidelines were twice as likely (71% vs. 36%, p=0.004) to achieve target drug exposure by 48 hours of therapy compared to those who did not. Vancomycin-associated AKI was observed in 9% of patients.

Discussion: Poor compliance with vancomycin guidelines was observed. Utilisation of dose prediction software provides an opportunity to correctly interpret all drug concentrations and individualise therapy to optimise patient outcomes.

Van Hal SJ et al (2013) Antimicrob Agents Chemother 57:734-44

Bio: Sophie Stocker is a Senior Hospital Scientist in the Department of Clinical Pharmacology and Toxicology at St Vincent's Hospital, Sydney. She is also a conjoint lecturer of the St Vincent's Clinical School, University of New South Wales. Her research program involves clinical and experimental pharmacology, ethnopharmacology, pharmacogenomics, pharmacometrics and qualitative research on the impact of intrinsic and extrinsic factors on drug disposition, efficacy and safety. Her research focuses on understanding variability in response to medicines and how this can be managed to optimise patient care.

Session 3: Rising Stars
Dr Ira Deveson

"Chiral DNA sequences as reference standards for clinical genomics"

2:30-2:50 pm

Abstract: Chirality is a geometric property describing any object that is inequivalent to a mirror image of itself. Due to its 5'-3' directionality, a DNA sequence is distinct from a mirrored sequence arranged in reverse nucleotide order, and is therefore chiral. A given sequence and its opposing chiral partner sequence share many properties, such as nucleotide composition and sequence entropy. Here we demonstrate that chiral DNA sequence pairs also perform equivalently during molecular and bioinformatic techniques that underpin modern genetic analysis, including PCR amplification, hybridization, whole-genome, target-enriched and nanopore sequencing, sequence alignment and variant detection. Given these shared properties, synthetic DNA sequences that directly mirror clinically relevant and/or analytically challenging regions of the human genome are ideal reference standards for clinical genomics. We show how the addition of chiral DNA standards to patient tumor samples can prevent false-positive and false-negative mutation detection and, thereby, improve diagnosis. Accordingly, we propose that chiral DNA standards can fulfill the unmet need for commutable internal reference standards in precision medicine.

Bio: Dr Ira Deveson is a Cancer Institute NSW Early Career fellow at the Garvan Institute of Medical Research, with a conjoint appointment at the University of New South Wales. Ira graduated from the Australian National University in 2012 and received the University Medal for Biology. During his postgraduate studies at the Garvan Institute (2014-2017), Ira worked on a variety genomics/transcriptomics projects, ranging from the development of diagnostic reference standards for clinical genomics, to an investigation of the molecular mechanisms underpinning reptile temperature-dependent sex determination. Ira's graduate research has resulted in 10 publications, including first author articles in Nature Methods and Science Advances, and he was recently awarded an Early Career Fellowship from CINSW to pursue his work on clinical reference standards for genomics.

Session 3: Rising Stars
Dr Charles Cox

"Using the force: Piezo channels as molecular reporters of mechanical forces"

2:50-3:10 pm

Abstract: Mechanosensitive channels are essential molecular components of mechanosensory systems in all organisms. The Piezo ion channel family is a recently discovered class of mechanosensitive channels found throughout eukaryotes. The family is comprised of two members Piezo1 and Piezo2. Piezo1 is found in many cell types throughout the cardiovascular system and is essential for vascular development. We have shown that Piezo1 possesses an innate ability to decode mechanical stimuli. However, this sensitivity is heavily influenced by the local cytoskeletal environment. Interestingly, these changes in Piezo1 sensitivity are not exclusively correlated to changes in bulk mechanical properties of the cell. Moreover, we find reciprocity in the fact that expression of Piezo1 profoundly affects the transcriptional landscape of cytoskeletal associated proteins. Our results shed light on the ability of different structural scaffold proteins to sensitize or desensitize Piezo1 channels to mechanical stimuli by modulating their tension sensitivity. This provides spatio-temporal means for the cell to fine tune its sensitivity to mechanical force.

Bio: Charles Cox is an NSW health EMC fellow at the Victor Chang Cardiac Research Institute (VCCRI). He completed his PhD at Cardiff University in the UK and subsequently joined the laboratory of Prof Boris Martinac. In 2015 he was awarded Young Biophysicist of the Year by the Australian Society of Biophysics. His work aims to understand how cells sense mechanical forces and in particular the molecular basis of force sensing in ion channels.

PLENARY: Prof Gemma Figtree

3:30-4:00 pm

Bio: Gemma Figtree is a Professor in Medicine at the University of Sydney, and Research Lead for Cardiothoracic and Vascular Health at the Kolling Institute and for Northern Sydney Local Health District. She co-leads the Cardiovascular Theme for Sydney Health Partners, a NHMRC Advanced Health Research and Translation Centre and is the Chair of the University of Sydney's multi-disciplinary Cardiovascular Initiative. Gemma completed her DPhil at Oxford University in 2002 supported by a Rhodes Scholarship and has continued in the field of oxidative signalling. She is committed to improving the care for heart attack patients- using her knowledge of redox signalling and molecular biology to develop methods of identifying those at highest risk of adverse outcome, and discovering novel therapies to prevent and treat events, inspired by her clinical work as an interventional cardiologist. Discoveries in her Laboratory have been published in leading journals Circulation, European Heart Journal, and FRBM, with > 130 publications. GF is a principal investigator on grants >$6.5 mill. Having recently completed a co-funded NHMRC CDF and Heart Foundation Future Leader Fellowship, she has been awarded a Practitioner Fellowship. She is committed to the advancement of her field, and serves as a member of the Editorial Board of leading international cardiovascular journals Circulation and Cardiovascular Research, as well as being a founding editorial board member for Redox Biology, and an Associate Editor for Heart, Lung and Circulation. Her research and clinical perspective and leadership are recognised by her membership of the Scientific Board of Cardiac Society of Australia and New Zealand (responsible for International Relations), and her appointment to the Expert Advisory Panel for NHMRC Structural Review of Grants Program (2016-17), and as well as the Clinical Issues Committee of the Heart Foundation. She is committed to the promotion and advocacy of cardiovascular research, recently appointed as President of the Australian Cardiovascular Alliance. She is a graduate of the Australian Institute of Company Directors, and serves/has served as a non-executive Director on multiple community Boards

FB RICE | PATENT & TRADE MARK ATTORNEYS

PATRICK MCMANAMNY has a PhD in Molecular Biology and Physiology. He has extensive experience assisting clients in achieving commercial objectives including large multi-nationals, start-up companies and research institutes. He has also served a brief period as in-house counsel, which has provided valuable insight into the responsibilities of this role.

Patrick regularly counsels clients in relation to freedom-to-operate issues, invalidity assessments, strategies for attacking third party rights and due diligence reports. He is sought out particularly for his knowledge and experience in patent issues relating to biological therapeutics.

Patrick is a registered patent attorney in Australia and New Zealand and a Partner at FB Rice.

KARIN INNES is a Senior Associate in the Biotechnology group with over 15 years' experience. She assists a broad range of clients in all facets of academia and industry with patent procurement and defense in Australia and New Zealand and internationally through her close network of foreign associate contacts.

Karin has a PhD from the prestigious Walter and Eliza Hall Institute for Medical Research, where her research focused on retroviral-based strategies for hematopoietic stem cell immortalisation. Her experience spans a number of different technology areas including molecular and cellular biology, gene cloning, gene expression and silencing, yeast biology, immunology, microbiology, intracellular signaling, virology, vaccines and CRISPR technology.

Karin is a registered patent attorney in Australia and New Zealand.

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Poster Abstracts:

Flash Talks and Fast Forward speakers with Posters

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7th Annual St Vincent's Campus PostDoc Symposium
Poster session
13th September 2018: 12:00-1:30 pm

P01: Tim Peters

Genome-wide bisulfite sequencing of rogue and memory B cells using scBS-Seq

Tim Peters[1], Manu Singh[1], Joanne Reed[1] and Christopher Goodnow[1]
[1]*Immunogenomics Laboratory, Garvan Institute of Medical Research, Darlinghurst, N.S.W. 2010*

We present initial results from a single cell bisulfite sequencing study profiling two groups of B cells isolated from a patient with Sjögren's syndrome. Half of the cells are from a "rogue" clonal expansion that recognises self-antigens via autoantibodies utilising the IGHV1-69 immunoglobulin variable region, and the other half are normal, polyclonal memory B cells. We want to characterise the differential methylation between the rogue and memory populations, and this obliges us to develop the most efficient bioinformatic processing pipeline for this end.

We display the bioinformatic pipeline used for the bisulfite sequencing assays and present inherent challenges and various strategies of recovering the maximum amount of information from them, particularly with respect to input and genomic resolution.

We present data characterising the trade-off between coverage and resolution with respect to summarising methylation states, and present examples where spurious differential calls may arise.
As with all single-cell assays, the dominant challenge with scBS-Seq is making robust inferences from the data despite an upper bound on the recovery of methylation calls. We touch on possible techniques for overcoming such limitations.

P02: Tim Peters

A general framework for evaluating cross-platform concordance in genomic studies

Timothy J. Peters[1], Terence P. Speed[2] and Susan J. Clark[1]
[1]*Epigenetics Laboratory, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia*
[2]*Bioinformatics Division, Walter and Eliza Hall Institute, Parkville, VIC 3052, Australia*

The reproducibility of scientific results from multiple sources is critical to the establishment of scientific doctrine. However, when characterising various genomic features (transcript/gene abundances, methylation levels, allele frequencies and the like), all measurements from any given technology are estimates and thus will retain some degree of error. Hence defining a "gold standard" process is dangerous, since all subsequent measurement comparisons will be biased towards that standard.

In the absence of a "gold standard" we instead empirically assess the precision and sensitivity of a large suite of genomic technologies via a consensus modelling method called the row-linear model. This method is an application of the American Society for Testing and Materials Standard E691 for assessing interlaboratory precision and sources of variability across multiple testing sites. We analyse a publicly available TCGA dataset containing both sequencing and array technologies, allowing a direct per-technology, per-locus comparison of sensitivity and precision across all common loci.

We implement and showcase a number of applications of the row-linear model, including direct comparisons of the sensitivity and precision of these platforms. Our findings demonstrate the utility of the row-linear model in evincing varying levels of concordance between measurements on these platforms, serving as a process for identifying reproducibility caveats in studies where cross-platform validation is performed.

P03: Rosemary Kirk

Investigating *Slc6a19* loss-of-function mutations as a cause of embryo loss and congenital defects in mice

Victor Chang Cardiac Research Institute

Congenital defects and recurrent miscarriage are common and devastating conditions, and in most cases their cause is unknown. Recently, Dunwoodie and colleagues demonstrated that mutations in two genes required for NAD synthesis from tryptophan cause these conditions in humans and mice. This raises the question of whether NAD-deficiency caused by other genetic mutations can have the same effect. To address this, we are investigating the gene *SLC6A19*, which encodes the major tryptophan transporter in the kidneys and intestines, and therefore plays an integral role in NAD synthesis by supplying this NAD-precursor to the body. We aim to determine whether mutations in *Slc6a19* cause miscarriage and congenital defects in mice.

In this study, *Slc6a19*$^{+/-}$ mice were mated, and pregnant females placed on diets deficient in NAD-precursors (accounting for the high metabolic rate of mice). After 18 days the mothers were culled, and the incidence of miscarriages and congenital defects assessed. Embryos were phenotyped by light microscopy, skeleton staining, and optical projection tomography. Statistical analysis was performed using a series of Fishers' exact tests.

Compared to mothers on the same restricted diet but without genetic mutation, *Slc6a19*$^{+/-}$ mice incurred greater frequency of embryo loss (70.18% v 12.16%, $p<0.0001$). This was significantly reduced through niacin supplementation (9.52%, $p<0.0001$). A broad range of defects were seen, however frequency was not significantly different from that in embryos of non-mutant mothers.

This study has identified *Slc6a19* loss-of-function mutation as a further NAD-related genetic cause of embryo loss, and a simple dietary supplement as a preventative method.

P04: Justin Szot

A Screening Approach to Identify Clinically Actionable Variants Causing Congenital Heart Disease in Exome Data

Victor Chang Cardiac Research Institute

Background: Congenital heart disease (CHD), structural abnormalities of the heart that arise during embryonic development, is the most common inborn malformation, affecting ≤1% of the population. However, currently, only a minority of cases can be explained by genetic abnormalities. The goal of this study was to identify disease-causal genetic variants from whole-exome data in 30 families affected by CHD.
Methods: We utilized a 2-tiered whole-exome variant screening and interpretation procedure. First, we manually curated a high-confidence list of 90 genes known to cause CHD in humans, identified predicted-damaging variants in genes on this list, and rated their pathogenicity using American College of Medical Genetics and Genomics-Association for Molecular Pathology guidelines.
Results: In 3 families (10%), we found pathogenic variants in known CHD genes TBX5, TFAP2B, and PTPN11, explaining the cardiac lesions. Second, exomes were comprehensively analyzed to identify additional predicted damaging variants that segregate with disease in CHD candidate genes. In 10 additional families (33%), likely disease-causal variants were uncovered in PBX1, CNOT1, ZFP36L2, TEK, USP34, UPF2, KDM5A, KMT2C, TIE1, TEAD2, and FLT4.
Conclusion: The pathogenesis of CHD could be explained using our high-confidence CHD gene list for variant filtering in a subset of cases. Furthermore, our unbiased screening procedure of family exomes implicates additional genes and variants in the pathogenesis of CHD, which suggest themselves for functional validation. This 2-tiered approach provides a means of (1) identifying clinically actionable variants and (2) identifying additional disease-causal genes, both of which are essential for improving the molecular diagnosis of CHD.

P05: Jihan Talib

Concurrent metabolomic-proteomic analyses of atherosclerotic lesions from a single mouse arterial segment

Jihan Talib,[1] Peter G Hains,[2] Philip J Robinson ,[2] Mark Hodson,[1] and Roland Stocker[1]

[1]Victor Chang Cardiac Research Institute, Sydney, Australia and [2]Childrens Medical Research Institute, Sydney, Australia

Background: The apolipoprotein E gene-deficient (*Apoe*$^{-/-}$) mouse serves as an essential tool in elucidating the pathophysiology of atherosclerosis due to its propensity to spontaneously develop arterial lesions. To date, however, an integrated omics assessment of atherosclerotic lesions in individual *Apoe*$^{-/-}$ mice has been challenging due to the small amount of diseased and non-diseased tissue available.
Methods: To address this current limitation, we are aiming to develop an integrated metabolomic and proteomic method that utilizes the Barocycler 2320EXT for tissue homogenization and digestion, coupled with high sensitivity mass spectrometry to assess arterial tissue from *Apoe*$^{-/-}$ mice fed a Western Diet for 3 months. Untargeted LC/MS based metabolomics, lipid profiling and proteomics will be conducted using the Sciex TripleTOF® 6600 mass spectrometer. Targeted metabolomics analyses will utilize the Agilent Metabolomics dMRM method for 215 metabolites.
Results: Our preliminary analyses show that from aqueous extracts of a single segment of mouse arterial lesion (~1 mg tissue) we can detect up to 80 metabolites and 4,000 features from the Agilent Metabolomics dMRM method and untargeted metabolomics respectively. The proteomics analyses indicate that we can quantify around 1,000 proteins.
Discussion: We will apply this method to compare metabolomics and proteomic profiles of lesion tissue from *Apoe*$^{-/-}$ mice to non-lesion tissue from age- and sex-matched C57BL/6J mice. If successful, our method of simultaneous profiling of metabolites and proteins may also be applicable to other situations where only small and valuable tissue samples are available, such as biopsy materials.

P06: Kenny Ip

Coordinated activation of amygdala - arcuate nucleus NPY circuits are required for the development of stress-induced obesity

Chi Kin (Kenny) Ip[1], Lei Zhang[1,2], Aitak Farzi[1] , Ireni Clarke[1], Felicia Reed[1], Yan-Chuan Shi[1,2], Ronaldo Enriquez [1], Yue Qi[1], Ramon Tasan[3], Günther Sperk[3] and Herbert Herzog[1,2,*]

[1]Neuroscience Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW, 2010, Australia, [2]Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia, [3]Institute of Pharmacology, Medical University Innsbruck, 6020 Innsbruck, Austria.

Neuropeptide Y (NPY) is one of the most powerful orexigenic peptides, exerting critical feeding related functions in the hypothalamus. However, NPY is also present in extra-hypothalamic nuclei where it is modulated in response to metabolic and other physiological perturbations, but far less is known about these NPY populations and their influence on energy homeostasis. Under conditions of high-fat-diet (HFD) feeding in combination with chronic stress (HFDS), the expression of NPY is robustly upregulated in the central amygdala (CeM) as well as in the arcuate nucleus (Arc), accompanied by an increase in HFD consumption resulting in a significant increase in body fat mass, and a decrease in energy expenditure (EE). To delineate the functional role of CeM-NPY under HFDS condition, we overexpressed NPY specifically in NPY neurons of the CeM in mice. This resulted in increase in food intake and diet-induced EE, subsequently leading to an increase in body fat mass. Importantly, specific ablation of NPY in the neurons of the CeM significantly reduced the obesity-associated phenotype, confirming the importance of NPY in the CeM for the regulation of stress-induced obesity (SIO). Not surprisingly, HFDS-treated mice are also less insulin sensitive with strongly reduced glucose tolerance. Under unstressed situation, insulin reduces the level of NPY in the CeM, which leads to the reduction in food intake in mice, exhibiting an anorectic action. However, our results show that this anorectic action of insulin on the CeM-NPY neurons is impaired under HFDS condition, exaggerating the development of diet-induced obesity. In summary, our data provide important new insights to the contribution of CeM-derived NPY in stress-induced feeding and also identifies the underlying mechanism for the development of obesity under combined high caloric food intake and stressful conditions.

P07: Ben William Johnson

Telmisartan Induces the Expression of UCP1 and Enhances Cellular Respiration in Human-Derived Adipocytes

Garvan Institute of Medical Research

Telmisartan, an angiotensin II receptor blocker, has been shown to augment the mRNA and protein expression of UCP1 in brown adipose tissue of rodents; which is associated with a reduction in weight gain, hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia. These findings are indicative of telmisartan's potential to be re-purposed for the treatment of obesity and diabetes. Despite this, telmisartan's adipocyte browning capacity in a human-based model is yet to be elucidated. Therefore the aim of this present study was to assess telmisartan's ability to induce and retain the mRNA and protein expression of UCP1 in primary adipocyte cultures isolated from human peri-thyroidal and subcutaneous supraclavicular adipose tissue. Additionally this study aimed to determine telmisartan's capacity to enhance the metabolic function of the primary adipocytes, such as cellular respiration. Preadipocytes were isolated from human supraclavicular adipose tissue and differentiated to produce either mature white or brown adipocytes. The mature adipocytes were treated with a 10μM dose of telmisartan for a duration of 7 days. RNA and protein was isolated from the adipocytes and subjected to real-time PCR and western blot analyses, respectively, to measure the expression of UCP1. Additionally, day 7 telmisartan-treated adipocytes were subjected to the Seahorse assay to measure mitochondrial oxygen consumption rate. Telmisartan was found to induce and retain the mRNA and protein expression of UCP1 in both peri-thyroidal- and subcutaneous-derived adipocytes, which was accompanied by an increase in mitochondrial oxygen consumption. These results indicate that telmisartan is capable of stimulating and retaining a brown thermogenic program in human adipocytes, which warrants further exploration for its potential use as an anti-diabetic drug.

P08: Alvaro Gonzalez-Rajal

Overcoming Platinum Resistance in Lung Adenocarcinoma: a live-imaging approach

Alvaro Gonzalez Rajal [1], Rachael A. McCloy [1], Max Nobis [1], Venessa Chin [1], Paul Timpson [1], Jason E. Cain [2], Andrew Burgess [1,3], D. Neil Watkins [1]

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3. *Microscopy and Flow Facility, ANZAC Research Institute, Concord, NSW 2139, Australia*

Background: Lung adenocarcinoma is the most prevalent lung cancer subtype. Platinum-based chemotherapy is the standard of care and although is initially effective the tumour becomes resistant in most cases and relapses, leading to therapeutic failure and patient death. Understanding lung adenocarcinoma's innate resistance to platinum would allow us to find new molecular targets that could be used in combined therapies to increase successful responses rates.

Methods: We have developed an *in vitro* model where lung adenocarcinoma cells are treated with cisplatin in a way that closely resembles its pharmacokinetic properties in solid tumours *in vivo*. Responses in a variety of cell lines with different *TP53* status was measured by analysing cell growth, population, cell cycle and DNA repair dynamics with live imaging, FACS and lineage tracing. *In vivo* studies in mouse have been done to confirm our *in vitro* results.

Results: We show that platinum resistance is a complex process in which most cells respond by undergoing permanent cell cycle arrest and are then replaced by replication competent cells, such that the total tumor burden appears unchanged. This response is seen in several lung adenocarcinoma cell lines, but in each case, cell cycle and DNA repair mechanisms vary according to *TP53* status.

Discussion: Our data directly challenge the prevailing model of platinum chemoresistance in lung adenocarcinoma, with major implications for a large number of patients. Patients could be stratified according to their *TP53* status to be treated with different combination agents to optimize platinum efficacy and minimize toxicity.

26th Annual St Vincent's Campus Research Symposium
Poster session
14th September 2018: 12:50-2:10 pm

P27: William Lee

Novel Pre-clinical Risk Prediction of Acquired Long QT Syndrome

William Lee[1,2], Melissa M. Mangala[1], Monique J. Windley[1,2], Matthew Perry[1,2], Jamie Vandenberg[1,2] and Adam Hill[1,2].

1. *Victor Chang Cardiac Research Institute*
2. *St. Vincent's Clinical School, UNSW*

Background: Acquired long QT syndrome (aLQTS) is caused by off target drug block of the Kv11.1 potassium channel resulting in delayed cardiac repolarisation, arrhythmias and sudden cardiac death. aLQTS is one of the primary reasons for the premature termination of drugs in the preclinical phase of development. However, we currently do not have a test for proarrhythmia that is both sensitive and specific.

Objective: To develop a novel predictive tool for drug induced arrhythmogenesis based on quantitative analysis of the morphology of cardiac action potentials

Methods: Ventricular myocytes, either from *in silico* simulations or recorded from induced pluripotent stem cell (iPSC) derived cardiomyocytes *in vitro,* were subjected to a panel of drugs with known variable risk profiles. A high throughput optical voltage sensing platform was used to acquire, iPSC cardiomyocyte action potentials (AP). Resultant AP morphologies were characterised by mathematical analysis of the morphology of the waveform using curvature analysis.

Results: *In silico* models demonstrated a dose dependent increase in the positive area under the curve of the curvature signal (R_{AUC}) for intermediate and high-risk proarrhythmic drugs. Moreover, increased R_{AUC} also predicted the emergence of early after depolarisations, and could be used to accurately identify low-risk drugs. R_{AUC} showed a similar dependence on concentration of proarrhythmic drugs in action potentials recorded from IPSC cardiomyocytes.

Conclusion: Quantification of AP morphology using *curvature analysis* can predict arrhythmogenic risk and has potential as the basis for new preclinical screens for proarrhythmia.

P28: Pietro Ridone

Cholesterol-dependent PIEZO1 clusters are essential for efficient cellular mechanotransduction

P. Ridone[1,*], E. Pandzic[2,*], M. Vassalli[3], C. D. Cox[1,5], A. Macmillan[2], P.A. Gottlieb[4] and B. Martinac[1,5]

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The human mechanosensitive ion channel PIEZO1 is gated by membrane tension and regulates essential biological processes such as vascular development and erythrocyte volume homeostasis. Currently, little is known about PIEZO1 plasma membrane localization and organization. Using a PIEZO1-GFP fusion protein, we investigated whether cholesterol enrichment, depletion (methyl-β-Cyclodextrin; MBCD) and the disruption of membrane cholesterol organization (Dynasore) affects PIEZO1's response to mechanical force. STORM super-resolution imaging revealed that, at the nano-scale, PIEZO1 channels in the membrane associate as clusters which increase in number upon cholesterol addition. Both cluster size and diffusion rates were profoundly affected by treatment with MBCD (5 mM). In addition, electrophysiological recordings in the cell-attached configuration revealed that MBCD caused a rightward shift in the PIEZO1 pressure-response curve and increased channel latency in response to mechanical stimuli. Our results indicate that PIEZO1 function is directly dependent on the amount and lateral organization of membrane cholesterol which is crucial for the concerted inactivation of PIEZO1 clusters.

P29 Aileen Chen

The Effectiveness of an Online Self-Guided Mindfulness Program for Psychological Wellbeing

Aileen Z. Chen[1], Natalie Klatnitski[1], Jill M. Newby[2]
[1]*Clinical Research Unit for Anxiety and Depression (CRUfAD), UNSW at St Vincent's Hospital.*
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Background
Face-to-face mindfulness-based treatments are effective, but not always easily accessible. Evidence suggests that mindfulness may be learnt by self-help, however less is known about the effectiveness of mindfulness interventions delivered over the internet. We aimed to evaluate the effectiveness of our free online *Intro to Mindfulness* course on levels of psychological distress and wellbeing in a non-clinical general population. A secondary aim is to investigate whether demographic factors would predict adherence to this online course.
Methods: 1355 members of the general public who self-selected to enrol in this course on ThisWayUp.org.au are included in this study as a part of the quality assurance activities of ThisWayUp. Each of the four lessons teaches a set of mindfulness skills, exercises through illustrated comic-style slides delivered over a 90-day period. Linear mixed models analysed changes in the Kessler-10 (K-10; distress) and Short Warwick-Edinburgh Mental Well-being Scale (SWEMWBS; wellbeing). We used chi-square and t-tests to compare the completer and non-completer subsamples on demographics and clinical variables, to determine factors associated with adherence.
Results: There were small to moderate significant improvements in distress (Hedge's g= 0.52) and wellbeing (Hedge's g= 0.37). No significant differences were found between the completers and non-completers on demographic and clinical variables, except for age, whereby older adults were more likely to complete.
Discussion: To our knowledge, this is the largest effectiveness study of an online mindfulness-based course. Of note, our course is short, accessible, and has great potential to improve psychological distress and wellbeing in the general population.

P30: Kazuo Suzuki

Evaluation of automated Nucleic Acid Extraction system from Buccal Swab samples

K. Grassi[1], B. Fsadni, K. Merlin, P. Cunningham[2], K. Suzuki[2*]
[1]HIV Clinical Trials, St Vincent's Centre for Applied Medical Research (AMR). [2]NSW State Reference Lab HIV, St Vincent's Centre for Applied Medical Research (AMR)

Background: Achieving the maximum yield and purity in DNA isolation from various clinical specimens provides an important foundation for clinical research based on molecular and diagnostic analyses. We had an opportunity to assess a method of DNA extraction from Isohelix SK-1S buccal swab samples for human genetic analysis with our collaborators from the School of Psychiatry, UNSW. Due to the nature of the sampling method, some of the the samples were found to contain bacterial DNA.
Methods: We conducted DNA extraction using the Maxwell automated system (Promega). After the extraction we used several methods to assess the extracted DNA: i) standard absorbance at 260nm using an Implen NanoPhotometer, ii) Qubit DNA procedure (Life Technology), which gave the specific DNA yield, and iii) human GAPDH DNA method with Real-Time PCR analysis.
Results: DNA extracted levels, based on i) the standard absorbance method and ii) Qubit DNA procedure, resulted in a relatively good yield of DNA across all samples. However, we found that the amount of human DNA varied across samples, with some showing very low yields.
Discussion: The data suggests that analysis of the presence of human DNA by simple GAPDH detection is important when assessing DNA extraction. Since buccal swab samples, by their very nature, will contain bacteria, we have recommended that GAPDH Real-Time PCR analysis be performed, before starting expensive down stream applications such as NGS.

P31: Matthew Perry

Exploring use of potassium channel activators to correct abnormalities in human pluripotent stem cell-derived cardiomyocyte models of congenital long QT syndrome

Matthew Perry[1,2], Adam Hill[1,2], Jamie Vandenberg[1,2]
[1]Victor Chang Cardiac Research Institute. [2]St. Vincent's Clinical School, UNSW

Background: Congenital Long QT syndrome type 2 (LQTS2) is an electrical disorder of the heart that predisposes individuals to life threatening cardiac arrhythmias. Current treatments either reduce the incidence of arrhythmia triggers or terminate the arrhythmia after onset, but are associated with significant side effects. One alternative strategy is to target the underlying cause: a loss of function of the human ether-a-go-go related gene (hERG) potassium channel. Small molecule activators of hERG have been identified but their potential to restore normal cardiac signaling in LQTS2 remains unclear.
Methods and Results: We characterized a human induced pluripotent stem cell derived cardiomyocyte (iPSC-CM) model of LQTS2 containing an expression defective hERG mutant A422T. Using a Kinetic Imaging Cytometer that records optical signals for both membrane voltage and intracellular calcium, we show that mutant A422T dramatically prolongs ventricular action potential duration and alters calcium handling properties. The emergent electrical phenotype of a monolayer (pseudo-tissue) of A422T iPSC-CMs was examined using an automated microelectrode array system. Mutant A422T dramatically prolonged the rate corrected field potential duration, a surrogate of QT interval on an electrocardiogram. The hERG activator ICA-105574 was able to reverse the field potential prolongation associated with A422T iPSC-CMs in a concentration dependent manner. However, at higher doses ICA-105574 produced a profound shortening of the field potential duration compared to controls.
Conclusion: We conclude that hERG activators could provide a possible treatment option for LQTS2 that targets the primary disease mechanism, with the caveat that there may be a risk of overcorrection that could itself be pro-arrhythmic.

P32: Wei How Lim

Trends and Management of Ruptured Ovarian Cysts

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St Vincent's Hospital, Sydney NSW
Institute of Minimally Invasive Surgery, St Vincent's Private Hospital, Sydney
St Vincent's Clinical School, UNSW Sydney

Background: Ruptured ovarian cysts are common gynaecological presentation to health institutions with abdominal pain. While this phenomenon is generally self-limiting, surgery may be necessary in cases of haemodynamic compromise or association with torsion. The aim of this audit is to identify the trend of hospital presentations, as well as the review the management of modern gynaecology practice.
Methods A retrospective audit of all women who presented to the emergency department with an imaging diagnosis of ruptured ovarian cysts was conducted over an 8 year period at St Vincent's Hospital, Sydney.
Results Preliminary findings of over 200 women revealed an upward trend in acute hospital presentations over the last 5 years. Majority of women were diagnosed with a ruptured corpus luteum and were managed conservatively. 20% of women required surgical intervention, with ovarian cystectomy performed in 70% of such cases, followed by diathermy to the ovary. As expected, women who had surgical intervention were more likely to have larger cysts or large haemoperitoneum findings on imaging compared to those managed conservatively. There were no statistically significant differences in location of ovarian cysts (right or left) or being on hormonal supplements (OCP or IUCD).
Discussion Ruptured ovarian cysts remained a common clinical presentation of acute pain for women of reproductive age and our audit suggests that the incidence is on the rise. This may be due to better availability and access to diagnostic imaging services with trained sonographers and radiologists. A high percentage of women had unpredictable menstrual cycle history which may have prompted imaging investigations when in casualty. Surgical management with laparoscopy was largely feasible with minimal complications.

P33: Cecilia Chambers

Inhibition of the NPY signalling axis as a novel therapeutic option in Pancreatic Cancer.

Chambers, C.[1], Herrmann, D.[1], Murphy, K.[1], Melenec, P.[1], Reed, D.[1], Zhang, L.[1], Enriquez R.F.[1], Shi Y.C.[1], Schofield P.[1], Pinese M.[1], Waddell N.[3], Christ D.[1], Morton, J.P.[2], Herzog, H.[1], Timpson, P.[1]

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Pancreatic cancer (PC) is currently the fifth leading cause of cancer-related deaths in Australia. The limited efficacy of current therapies and the presence of advanced disease at the time of diagnosis has resulted in a 5-year survival of less than 8% and this has changed very little in the last 40 years highlighting the need for better treatment options for PC. Analysis of the Australian Pancreatic Genome Initiative (APGI) patient cohort showed that 16% of patients displayed amplification of Neuropeptide Y (NPY) signalling components. NPY is the most abundant neuropeptide in the central nervous system, and is involved in numerous processes including energy and bone homeostasis and also cancer progression. Preliminary data has shown significantly reduced tumour growth in Lewis Lung Carcinoma and B16F10 Melanoma upon genetic or pharmacological inhibition of NPY signalling, leading us to investigate the role NPY plays in PC. Here, we show in our well-established mouse model of PC, which is driven by mutations in *Kras* and *p53*, that are also commonly mutated in human PC that: (i) NPY RNA and protein expression is up-regulated in primary tumours and metastases compared to wildtype pancreas (ii) Inhibition of NPY signalling *in vitro* with a small molecule inhibitor reduces PC cell migration, cell streaming and cell cycle progression. Experiments to block NPY in our *in vivo* mouse models and in patient-derived models of PC are currently ongoing, which may inform future applications.

P34: Daniel Reed

New tools to assess cancer spread and anti-invasive efficacy

Daniel Reed[1], Sean Warren[1], Max Nobis[1], Pauline Melenec[1], David Gallego-Ortega[1], Aurelie Cazet[1], Douglas Strathdee[2], Jody Haigh[2], Zahra Erami[3], Kurt I. Anderson[3], David Herrmann[1], Paul Timpson[1].

[1]The Garvan Institute of Medical Research & The Kinghorn Cancer Centre, [2]Monash University, [3]Cancer Research UK Beatson Institute.

Background: E-cadherin-mediated cell-cell junctions play a prominent role in maintaining epithelial architecture. Their dysregulation in cancer can lead to the collapse of tumour epithelia and subsequent invasion and metastasis. Recent evidence suggests that, apart from modulating E-cadherin expression, cells are able to mobilise E-cadherin within their cell-cell junctions upon migration and invasion. We have developed new tools to assess the spatiotemporal dynamics of epithelial tumour cell-cell junctions to study the earliest stages of invasion and metastasis.

Methods: Here, we have generated an E-cadherin-GFP mouse, which enables intravital quantification of E-cadherin clustering and mobility to provide insight into tumour cell-cell junction strength and integrity in intact tissues and tumours.

Results: We reveal that: (1) E-cadherin mobility and clustering become de-regulated in invasive and metastatic tumours compared to healthy tissues and non-invasive pancreatic tumours. (2) These subcellular aberrations in E-cadherin dynamics can be targeted with antiinvasive treatment to re-stabilise cell-cell junctions and to reduce cancer invasiveness.

Discussion: We suggest that these techniques can be used as: (1) **novel tools** to fundamentally expand our understanding of cell-cell junction dynamics *in vivo* in native microenvironments (2) **novel pre-clinical drug-screening platform** to predict cancer spread and to estimate the efficacy of anti-invasive treatment.

P35: Sean So

Identifying small molecule ligands for the orphan G protein-coupled receptor, GPR37L1

Sean S So[1,2], Tony Ngo[3], Brendan P Wilkins[1,2], Andrey V Ilatovskiy[3], Marcello Leopoldo[4], Irina Kufareva[3], Nicola J Smith[1,2]

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Background: We developed GPCR-CoINPocket (GPCR-Contact-Informed Neighbouring Pocket), a crystal structure-informed metric of GPCR similarity, enabling prediction of off-target ligand activities between deorphanised and orphan GPCRs. We applied GPCR-CoINPocket to identify 3 novel inverse agonists of the blood pressure-modulating orphan, GPR37L1 (30% hit rate). However, some known pharmacological GPCR similarities were not recapitulated, potentially because the original GPCR-CoINPocket utilised calculations that did not directly measure residue similarity based on ligand-binding characteristics. We aim to improve the accuracy of GPCR-CoINPocket by incorporating fundamentally critical extracellular loop contacts and accounting for ligand-binding characteristics in the calculations, and to identify ligands for orphan GPCRs.

Methods: GPCR-CoINPocket was updated by refreshing the source crystal structure database and including critical extracellular loop contacts. GPCR-CoINPocket2.0-based relationships were calculated for all GPCRs. Experimental validation was performed using reporter gene assays and is ongoing. Work to rewrite the residue similarity matrix to directly compare residue similarity with respect to ligand-binding characteristics to further improve the metric is ongoing.

Results: Recalculating the GPR37L1 neighbourhood revealed unexpected aminergic neighbours. Our 4 GPR37L1 surrogate inverse agonists display varying off-target activity at $\beta_{1/2}$ adrenoceptors in cAMP reporter assays, validating GPCR-CoINPocket2.0.

Discussion: Predicting pharmacological similarities between deorphanised and orphan GPCRs guides the identification of novel surrogate ligands. These ligands enable the elucidation of orphan GPCR function and facilitate further drug development via parallel *in vitro* and *in silico* approaches. GPCR-CoINPocket is applicable to all proteins and orphan GPCRs. Improving the metric allows identification of novel surrogate ligands to further power investigations into orphan GPCRs.

P36: Matthew Peck

Using T wave morphology to predict proarrhythmic risk in drug induced arrhythmia

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Drug-induced or acquired LQTS (aLQTS) is caused by pharmacological block of the human ether-a-go-go-related gene (hERG) potassium channel and can develop into the potentially fatal ventricular arrhythmia torsades de pointes. The QT interval derived from an electrocardiogram (ECG) is the most commonly used clinical measure to predict drug induced arrhythmias, however previous work has shown that while sensitive, it is a non-specific biomarker for risk. Alternatively, morphological changes in the T wave have recently been shown to have promise as more accurate predictors of arrhythmia. However, the link between ECG waveform morphology and underlying pharmacology, and the potential of morphological measures as new biomarkers of risk remains to be fully described. In this study, ECG data from 22 patients, each administered four different drugs (Dofetilide, Quinidine, Ranolazine and Verapamil) were analysed using an automated signal processing approach developed in house. Briefly, the principal component of the ECG T-wave was extracted from the precordial leads and fitted with a combination of 3 sigmoid functions (upslope, downslope and switch) and two 9^{th} order polynomial functions (upslope and downslope). Linear mixed effect models were used to identify parameters with significant dose dependence. Time constants from sigmoid fits, together with polynomial coefficients were used in machine learning algorithms to identify multichannel pharmacology and predict risk. Sigmoid fit functions (upslope, downslope and switch), along with upslope polynomial coefficients (U0, U1, U2 and U3) demonstrated significant dose dependency for unopposed hERG block, whereas drugs that have multichannel blockade showed a reduced dose-dependency correlation. Using morphological parameters fitted to ECGs recorded at baseline, we aim to predict patients who are particularly susceptible to drug induced QT prolongation.

P37: Rhyll Smythe

Impaired relaxation of resistance arteries isolated from orphan receptor GPR37L1-deficient female mice in response to a nitric oxide donor

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Background: Orphan G protein-coupled receptors (GPCRs) are GPCRs yet to be matched with endogenous ligands. Such orphan GPCRs represent a large potential resource of new drug targets. GPR37L1 is an orphan receptor expressed in the brain and established by our laboratory to increase blood pressure when deleted in female mice. When deleted in male mice, it causes heightened left-ventricular hypertrophy in response to angiotensin II infusion. We aimed to resolve the mechanism by which female GPR37L1 null mice ($GPR37L1^{KO/KO}$) have increased blood pressure compared to wildtype counterparts ($GPR37L1^{wt/wt}$). We hypothesise there is a genotype-dependent difference in responsiveness of resistance blood vessels to physiological mediators of vessel constriction and dilation.
Methods: Using wire myography, we assessed the response of resistance 3rd order mesenteric arteries from female $GPR37L1^{KO/KO}$ and $GPR37L1^{wt/wt}$ mice, to the endogenous vasoconstrictor, noradrenaline, and the nitric oxide donor and vasodilator, sodium nitroprusside (SNP). Data were analysed with extra sum-of-squares F test, n= 6-7.
Results: Vessels isolated from $GPR37L1^{wt/wt}$ mice exhibited a lower $logEC_{50}$ in response to SNP, compared to arteries from $GPR37L1^{KO/KO}$ mice (-7.622M vs -6.796M respectively, $p<0.05$). Vessel response to noradrenaline was comparable between genotypes.
Discussion: The lower $logEC_{50}$ exhibited by the wildtype vessels suggests that GPR37L1 deletion confers increased resistance to SNP-induced arterial relaxation when tested *ex vivo.* SNP acts by liberating the potent vasodilator, nitric oxide, without involving nitric oxide synthase. Therefore, the elevated blood pressure observed in female $GPR37L1^{KO/KO}$ mice may be due to dysfunction of dilatory pathways modulated by nitric oxide, and independent of nitric oxide synthase activity.

P38: Thao Phuong Ho-Le

CLINICAL UTILITY ASSESSMENT OF GENETIC PROFILING IN FRACTURE RISK PREDICTION: A DECISION CURVE ANALYSIS APPROACH

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Aims: Genetic profiling is emerged as a promising tool for assessing the risk fracture in asymptomatic individuals. This study used a decision curve analysis approach to determine the utility of genetic profiling in prediction of fracture.
Methods: The study involved 2188 women and 1324 men aged 60 years and above who have been followed up to 20 years. Bone mineral density (BMD) and clinical risk factors were obtained at baseline. The incidence of fracture and mortality was recorded during the period. A weighted genetic risk score (GRS) was constructed for each individual from sixty-two BMD-associated genetic variants. Four models were considered: **Model I (Base model)** included only clinical risk factors (CRF); **Model II (Garvan model)** included CRF and femoral neck BMD; **Model III (Garvan+GRS)** included CRF, femoral neck BMD and GRS; and **Model IV (Base+GRS)** included CRF and GRS. Decision curve analysis was used to evaluate the clinical net benefit in terms of true positives and false positives of predictive models.
Results: In women, for risk threshold above 0.15, the Base+GRS model had a significantly higher net benefit than the Base model; however, for threshold below 0.15, there was no significant difference in net benefit between the two models. The Garvan model yielded the highest net benefit; adding GRS into the Garvan model did not substantially improve the net benefit. In men with fracture risk greater than 0.15, the Base+GRS model resulted in a better net benefit than the Base model. Interestingly, the Garvan+GRS model did improve the net benefit over and above the Garvan model.
Conclusion: Genetic profiling can provide additional prognostic information to that obtained from clinical risk factors, particularly in women at higher risk of fractures. However, in the presence of BMD in a predictive model, GRS does not further improve net clinical benefit.

P39: Min Li Huang

Case Report of Unusual ALK Gene Rearrangement and Review of 354 Cases of ALK Rearrangement Study of Lung Non-Small Cell Carcinoma (NSCLC).

St Vincent's Hospital Sydney

Dementia and osteoporosis are common among elderly with some studies suggesting a causal link. Longitudinal studies that assess the complex relationships among cognitive decline, bone loss and fracture risk independent of ageing are lacking.

We aimed to determine the association between: 1) cognitive decline and bone loss, and 2) significant cognitive decline (≥3 points) on Mini Mental State Examination (MMSE) (baseline (Y0) - Year 5 (Y5)) and fracture risk (Y5 - 15).

A cohort of 3287 women and men 65+ from population-based Canadian Multicentre Osteoporosis Study (CaM*os)* was followed for 15 years. Association between bone loss and cognitive decline was estimated using mixed-effects models, and fracture risk using Cox Proportional Hazards models. Models were adjusted for age, education and co-morbidities.

Over 95% of participants had normal cognition at baseline. Annual % change in MMSE was similar for women [-0.33 (IQR:-1.00 to +0.02)] and men [-0.33 (IQR:-0.72 to 0.00)]. After accounting for confounders, cognitive decline was significantly associated with bone loss (0.13%/year MMSE loss for each 1%/year BMD loss). Approximately 13% of participants experienced significant cognitive decline by Y5. In this group, fracture risk was increased only in women [HR, 1.45 (95% CI: 1.00 to 2.10)]. After adjustment for bone loss the magnitude decreased slightly [HR, 1.39 (95% CI, 0.93-2.06)].

This study showed a significant association between cognitive decline and bone loss. Women with significant cognitive decline experienced increased fracture risk, which was only marginally explained by bone loss. Further studies are needed to determine mechanisms that link these common conditions.

P40: Weiyu Chen

The role of biliverdin reductase-a and bilirubin in cardiovascular disease

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Bilirubin has antioxidant activities and these could conceivably contribute to the observed inverse association between plasma bilirubin and cardiovascular diseases (CVD) that are associated with oxidative stress. However, to date a causal link between plasma bilirubin and CVD remains to be established, just as direct evidence for bilirubin's antioxidant activity *in vivo* remains limited.

As bilirubin is formed from biliverdin by biliverdin reductase, we generated *biliverdin reductase-a* gene knockout ($Bvra^{-/-}$) mice to assess the contribution of bilirubin as an endogenous antioxidant, and its potential role on atherosclerotic plaque instability.

$Bvra^{-/-}$ mice are born in expected Mendelian ratios and appear normal. Compared to littermate $Bvra^{+/+}$ mice, bile of $Bvra^{-/-}$ mice is green and their plasma bilirubin concentration is ~100-fold lower, while plasma biliverdin is increased ~25-fold. $Bvra^{-/-}$ and $Bvra^{+/+}$ mice have comparable plasma lipid profiles and low-molecular weight antioxidants. However, $Bvra^{-/-}$ mice have higher concentrations of plasma cholesterylester hydroperoxides, and their erythrocyte peroxiredoxin 2 is more oxidized compared to controls. To assess the role of bilirubin in unstable atherosclerotic plaque, we employed the tandem stenosis model of plaque instability in *apolipoprotein-E* gene knockout ($Apoe^{-/-}$) mice. Deletion of *Bvra* significantly decreases fibrous cap thickness in unstable plaque as assessed histologically, compared with $Bvra^{+/+}Apoe^{-/-}$ mice. We also observed that $Bvra^{-/-}Apoe^{-/-}$ animals have elevated plasma lipids, enhanced endothelial dysfunction and increased atherosclerosis.

Current data indicate that the absence of *Bvra* and bilirubin increases systemic endogenous oxidative stress and destabilizes the atherosclerotic plaque.

P41: Angela Sheu

Fracture in Type 2 Diabetes Confers Excess Mortality

Garvan Institute of Medical Research

Background: Despite higher bone mineral density (BMD), type 2 diabetes mellitus (T2DM) may increase fracture risk. Both fragility fractures and T2DM independently increase mortality, but the mortality risk after a fracture in T2DM is unknown. We aimed to determine the fracture and post-fracture mortality risk in T2DM.

Methods: In the Dubbo Osteoporosis Epidemiology Study, the longest-running population-based osteoporosis study internationally, radiologically-confirmed fractures, BMD and date of T2DM diagnosis and comorbidities were collected from 1989-2017. Fracture and survival analyses were performed using time-to-event for fracture and T2DM diagnosis (censored at death).

Results: There were 272 participants with, and 3346 without, T2DM at recruitment. T2DM participants had higher BMI, BMD, prevalent fractures and comorbidities at baseline.

Over 43,215 person-years, there were 796 incident fractures in women (64 in T2DM) and 240 in men (27 in T2DM). T2DM did not increase fracture risk in either gender after multivariate adjustment.

After multivariate adjustment, mortality was increased in T2DM women (HR 1.45, $p=0.0004$) but not men (HR 1.09, $p=0.47$). Incident fracture was associated with increased mortality in both women (HR 1.94, $p<0.0001$) and men (HR 2.04, $p<0.0001$). Mortality was highest in T2DM with fracture (HR 2.30, $p<0.0001$ in women, HR 2.72, $p=0.0008$ in men).

Discussion: T2DM was associated with higher prevalent, but not incident, fractures. The combination of T2DM with fracture confers significantly elevated mortality risk. Thus, bone fragility may affect a subset of T2DM patients and methods to identify and treat those at risk are warranted given the severe consequences.

P42: Tamara Yael Milder

Combination therapy with an SGLT2 inhibitor as initial treatment for type 2 diabetes: a systematic review and meta-analysis

St Vincent's Hospital Sydney

Background: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) are potentially attractive agents for initial combination therapy with metformin for type 2 diabetes (T2DM), particularly because of their extra-glycaemic benefits.
Aims: To compare the efficacy and safety of combination SGLT2i/metformin with either metformin monotherapy, or SGLT2i monotherapy in treatment-naïve T2DM adults. To compare high dose and low dose SGLT2i combination with metformin.
Methods: We searched PubMed, EMBASE and Cochrane Library for randomised controlled trials (RCTs) of SGLT2i. RCTs were selected if they (1) enrolled treatment-naïve T2DM participants (2) compared combination therapy with an SGLT2i to monotherapy (each agent in the combination) (3) change from baseline in HbA1c, weight, and adverse events were reported.
Results: Four RCTs (n=3749) were eligible for inclusion. Combination SGLT2i/metformin resulted in a greater reduction in HbA1c (-0.55% [95% CI -0.67, -0.43]) and weight (-2.00 kg [95% CI -2.34, -1.66]) compared with metformin monotherapy after 24-26 weeks of treatment. Combination SGLT2i/metformin resulted in a similar HbA1c reduction (-0.59% [95% CI -0.72, -0.46]) but an attenuated weight reduction (-0.57 kg [95% CI -0.89, -0.25]) compared with SGLT2i monotherapy over the same treatment period. High dose SGLT2i/metformin combination resulted in no HbA1c difference (0.02% [95% CI -0.08, 0.13]) but greater weight reduction (-0.47 kg [95% CI -0.88, -0.06]) compared with low dose SGLT2i/metformin therapy.
Conclusion: Initial combination therapy with SGLT2i/metformin has HbA1c and weight benefits, compared with either agent alone. High dose SGLT2i/metformin combination therapy appears to have modest weight but limited glycaemic benefits compared with the low dose SGLT2i/metformin combination therapy.

P43: Vanessa Hang Lam Wan

The effect of hyperthermia (42 °C) on the anti-tumoral effect of bromelain, N-acetyl cysteine, chemotherapeutic agents and their combinations – an in-vitro evaluation.

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Introduction: Bromelain (Br) , N-acetyl cysteine (Nac) and their combination inhibit gastrointestinal cancer cells' proliferation and survival at 37 °C. The combination also enhance the cytotoxicity of common chemotherapeutic agents (gemcitabine, Mitomycin C, oxaliplatin and 5-FU). At the same time, hyperthermia (42 °C) is also known to inhibit neoplastic cellular proliferation and hence we are investigating if hyperthermia would further enhance the cytotoxic effects of these agents.
Method: Pancreatic and colorectal tumour cells were grown in 96-well plates and treated with Nac, Br and their combinations with chemotherapeutic agents at 37 °C and 42 °C. The survival of cells at 72-hour was evaluated with sulforhodamine B assay. Colony formation assay was performed to investigate the development of resistance to these agents and finally PAS staining was carried out to determine the effect of Br, NAC, oxaliplatin and their combinations on mucin secretion in ASPC-1 (pancreatic)cells.
Results: Hyperthermia (42 °C) on the whole did not show any enhancement of effect by most of the agents in these cell lines. Some effects were observed at specific concentrations of agents in certain cell lines. Colony formation was reduced with hyperthermia in CFPAC (pancreatic cancer) cells when treated with bromelain, NAC and their combinations with gemcitabine or 5FU. Hyperthermia reduced mucin secretion in ASPC-1 cells for all combinations of bromelain, NAC, oxaliplatin except when NAC is used alone.
Conclusion: Hyperthermia did not show a distinct advantage in enhancing the cytotoxicity of bromelain, N-acetyl cysteine, chemotherapeutic agents or their combinations in pancreatic and colorectal tumour cells, however, it reduced colony formation and the secretion of mucin.

P44: Dima Alajlouni

Role of individual components of sarcopenia in fracture risk prediction in elderly women and men

Garvan Institute of Medical Research

Background: The relationship between sarcopenia and fracture is controversial. We aimed to assess the independent contribution of individual components of sarcopenia (muscle mass, strength and performance) and their decline rate to fracture risk prediction.
Methods: The study involved 912 women and 502 men 60+ years from the longitudinal Dubbo Osteoporosis Epidemiology Study, who had a total body BMD scan and/or muscle strength and function measurements. Fractures were ascertained by X-ray report between 2000 and 2017. Clinical data, lean muscle mass (MM), BMD, quadriceps strength (QS), gait speed (GS), sit-to-stand (STS) and timed get-up-go (TGUG) were measured biannually. Cox proportional hazards models adjusted for age, BMI, prior fracture, falls, smoking, alcohol and co-morbidities were used to quantify the association between components of sarcopenia and their decline rate with fracture risk.
Results: There were 262 incident fractures in women and 79 fractures in men over a median of 10 years follow-up. In women, none of the sarcopenia components measured at baseline independently contributed to fracture risk. By contrast, in men, QS, STS, TGUG and GS were associated with increased fracture risk. The decline rate of QS, STS, TGUG and GS were associated with fracture risk in women but not in men. Interestingly, MM did not contribute to fracture risk in either women or men.
Discussion: In the sarcopenia framework, muscle function (muscle strength and performance) appears to be the major contributor to fracture risk and should be measured in addition to other risk factors in elderly people.

P45: Andrew McCulloch

Targeting of Akt signalling and DNA damage response in pancreatic cancer.

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Pancreatic ductal adenocarcinoma (PDAC) is a leading contributor to cancer-related mortality in Australia, with fewer than 8% of sufferers surviving 5-years beyond diagnosis. Poor response to standard-of-care gemcitabine + *nab*-paclitaxel underscores the need for novel therapeutic approaches to target this aggressive disease. Upregulation of the PI3K/Akt/mTORC oncogenic pathway is emerging as an attractive therapeutic target, present in over 21% of PDAC. PI3K stabilises DSB repair through its interactions with the homologous recombination (HR) complex, and is required for efficient DNA damage repair. Furthermore, deficiencies in HR DNA damage repair such as loss of *BRCA2* have emerged as promising therapeutic targets in 9-14% of PDAC patients featuring genomic instability. Combined targeting of these pathways has demonstrated synergism in a preclinical model of breast cancer.

Here, we explore the benefits of combination therapy with a PARP1 inhibitor, rucaparib, and a dual mTORC1/2 inhibitor, AZD2014 (vistusertib), in both *in vitro* and *ex vivo* models of PDAC. Assessment of drug efficacy *in vitro* reveals strong synergism between AZD2014 and rucaparib in *BRCA2*-deficient PDAC lines, and an expected increased susceptibility to rucaparib compared to *BRCA2*-competent lines. Moreover, heightened sensitivity to AZD2014 suggests *BRCA2*-deficienct cells may increase their reliance on PI3K/Akt signalling. Assessing the response of *BRCA2*-competent cells to these inhibitors in an organotypic assay, we demonstrate reduced invasion through the extracellular matrix upon AZD2014 monotherapy and increased DNA damage upon rucaparib treatment. Finally, using an Akt-FRET biosensor we can assess endogenous Akt activity in *ex vivo* murine tumours. We highlight alterations in Akt activity from early acinar-to-ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanINs) through to primary PDAC and metastases. This work underscores the therapeutic potential of rucaparib and AZD2014 in PDAC patients featuring DNA damage repair deficiencies.

P46: Celine Santiago

Does genetic predisposition to dilated cardiomyopathy increase susceptibility to chemotherapy-induced cardiotoxicity?

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Doxorubicin (DOX) is a highly effective cancer chemotherapy agent that is frequently associated with dose-dependent cardiac toxicity. Although a "safe" cumulative lifetime dose has been proposed (450mg/m^2), a subset of individuals will develop dilated cardiomyopathy (DCM) even at lower doses. We hypothesize that these individuals carry genetic variants that increase susceptibility to DOX-induced cardiotoxicity. Anecdotal observations in familial DCM kindreds in our laboratory suggest truncating variants in the *TTN* gene (*TTN*tv) may modify the risk of DOX-induced cardiotoxicity.

We have generated a zebrafish model of a human *TTN*tv and found that heterozygous mutation carriers spontaneously develop DCM in adult life. To investigate DOX effects, the cardiac function of wildtype and heterozygous mutant fish was assessed before and after intraperitoneal DOX treatment using high frequency echocardiography. Previous work in our lab has demonstrated that DOX treatment in wildtype fish results in significant impairment of ventricular contraction and increased end-diastolic volume (10µg/gram body mass: ΔEF -6.50, ΔEDV+7.26, $p < 0.05$ as assessed by paired Student's T-test) – the hallmarks of DCM. Assessment of genotype effect on DOX response is currently underway.

In conclusion, intra-peritoneal DOX injection causes dose-dependent DCM in adult zebrafish, which we hypothesise will be more severe in *TTN*tv carriers. Given the high population frequency of *TTN*tv and the increasing use of genetic testing, this gene-environment interaction has important clinical relevance and may inform cancer chemotherapy management in *TTN*tv carriers.

P47: Kaiming Luo

The role of Hmox1a in zebrafish development

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Precise control of iron homeostasis is essential for diverse cellular processes and its dysregulation causes fatal developmental defects. Iron metabolism is regulated by heme oxygenase 1 (Hmox1), which is induced during stress and protects cells from oxidative damage by degrading heme into carbon monoxide, biliverdin and free iron. We hypothesize that while having an important role in cellular antioxidant defence, Hmox1 also plays a novel role in the activation of a signal transduction pathway essential for pattern formation and differentiation during zebrafish embryogenesis. To address this hypothesis, we generated a mutant zebrafish strain harbouring a loss-of-function allele of *hmox1a*, an orthologue of Hmox1 in zebrafish. The mutant Hmox1a exhibited a severely reduced production of biliverdin and bilirubin from heme *in vitro*, suggesting the lack of enzymatic activity of the mutant Hmox1a. Zebrafish carrying homozygous *hmox1a* mutant alleles showed severe developmental defects, including a reduced notochord and missing floor plate as well as cyclopia, which is indicative of defects in prechordal plate mesoderm and reminiscent of those observed with mutant zebrafish lacking Nodal signalling. We are currently investigating genetic interactions between *hmox1a* and Nodal pathway genes.

P48 Michelle Xu

Using induced Pluripotent Stem Cell (iPSC) Models to Characterise the Role of Genetic and Environmental Factors in Variable Response to Proarrhythmic Drugs.

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A large number of potential therapeutics fail during development due to risk of fatal heart rhythm disturbances and cardiac toxicity. Therefore, the development of predictive *in vitro* assays for preclinical safety assessment is an important factor for drug development. Cardiomyocytes (CMs) derived from human induced pluripotent stem cells (iPSCs) hold significant promise for the improvement of these preclinical screening as they offer a more clinically relevant cell-based model than those currently available. However, one factor that has not yet been captured *in vitro* is the variable risk associated with individual drugs across populations of normal subjects. In this study we will use a panel of genetically diverse iPSC lines to quantify the contribution of genetic variability and environmental factors, specifically adrenergic stimulation, to variable response to proarrhythmic drugs. iPSCs were differentiated into cardiomyocytes using commercially available protocols. Variability in expression of key cardiac ion channel genes in our panel of cell lines were measured using nanostring *nCounter* technology and electrical and calcium handling phenotypes of each line in response to proarrhythmic drugs, in the presence and absence of adrenergic stimulation, were measured using Kinetic Imaging Cytometry (Vala Sciences, USA). We will use this data to quantify the role of genetic and environmental factors in determining risk profile across the normal population. The insights gained will lead to a better understanding of the mechanisms of drug induced arrhythmias to help develop better preclinical tests. In doing so we will also assess the utility of human iPSC-CMs in preclinical drug screening and discovery.

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