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100. Challenging Dogma: Time to Change Practice with Some New Tricks

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Introduction. Dogma is rampant in medicine: principles or sets of principles that have been laid down by experts in the field as incontrovertibly true. The source of the fact may be absent, obfuscated, misconstrued, outdated, or misstated. Despite this, the notion can be perpetuated in textbooks, in systematic reviews, in guidelines, and in practice.

Aims. This session provides examples of dogma in medicine, and introduces a novel approach to address dogma, WikiGuidelines.

Methods. Guideline topics, literature searches, and evidence summaries are crowd-sourced, and participants are authors from diverse practice settings (academics, clinicians, rural, urban, multidisciplinary, and from countries with a range of access to resources). Topics are answered with either 1) a clear recommendation or 2) a clinical review. A clear recommendation requires replicated (2 or more) hypothesis-confirming results from prospective, controlled, and/or randomized trials.

Table 3. Summary of Oral Transitional Antibiotics Used in Published Clinical Studies[a]

Drug	Organism
Amoxicillin	• Sensitive streptococci (with or without combination treatment) • Enterococci (only in combination with rifampin or linezolid)
Dicloxacillin	Sensitive staphylococci (data from RCT only in combination with rifampin)
Levofloxacin[b]	Sensitive staphylococci (only in combination with rifampin)
Moxifloxacin	Sensitive streptococci, enterococci, or staphylococci (only in combination with amoxicillin, rifampin, or linezolid)
TMP-SMX	Sensitive staphylococci
Linezolid	Sensitive gram-positive cocci (alone or in combination with rifampin, moxifloxacin, or amoxicillin)[c]
Rifampin	Only as adjunctive agent (as previously described)

Results. To date, two WikiGuidelines have been published with this novel approach, in JAMA Network Open. They addressed the diagnosis and management of osteomyelitis (Spellberg et al.,) and the diagnosis and management of endocarditis (McDonald et al.,). In the endocarditis guideline, of 17 questions addressed, only 1 had a clear recommendation (oral antimicrobial transition therapy can be used in the treatment of endocarditis; Figure).

Discussion. Guidelines may present strong recommendations based on weak evidence, which can perpetuate dogma in medicine. We created WikiGuidelines to standardize clinical care with the humility of uncertainty.

McDonald EG (2023) JAMA Network Open; Spellberg B (2022) JAMA Network Open.

200. GPCRomics: Oh, the Places You'll Go!

Dr Fiona Murray, University of Aberdeen – British Pharmacological Society keynote address

Our lab focuses on understanding how the second messenger cyclic AMP can be regulated in a tissue and cell specific manner. One approach we have utilised routinely in the lab is to identify and quantify G protein-coupled receptors (GPCRs) in patient derived cells and analyse how their expression and function changes with disease. Such data, which has defined "GPCR signatures" that could serve as a novel biomarker and/or therapeutic target, has taken our lab on a journey from pulmonary arterial hypertension to uncovering the physiological relevance of an orphan GPCR.

201. New therapeutics require new health economic modelling approaches

Prof Zanfina Ademi, Monash University

Symposium attendees in the first part of the session will be introduced to examples of health technology assessment processes with a focus on the effectiveness and cost-effectiveness of cardiovascular therapeutics. Followed by a second part where Prof Ademi will present the current limitations of pharmacoeconomic models and propose a new way to integrate the casual duration of exposure to the lifetime risk of cardiovascular disease. Prof Ademi then will conclude whether new pharmacoeconomic modelling approaches will improve prevention and provide a shred of better evidence for the allocation of healthcare resources.

202. The journey beyond the bench

Robert P Shepherd, Dimerix Bioscience, Fitzroy, VIC, Australia

Developing a new medicine from initial discovery through clinical development and eventual commercialisation is a journey that can take over a decade, cost over a billion dollars USD, and has a low probability of success (DiMasi et al., 2016). The majority of the time, cost and risk beyond the discovery phase will occur in the complex environment of clinical drug development in a small biotech company. The complexities of modern drug development programs in a small biotech require agile teamwork and clear communication to efficiently progress a candidate molecule towards the clinic. The development of a small-molecule chemokine pathway inhibitor for the treatment of a rare fibrotic kidney disease will be used as an example of navigating the journey from proof-of-concept studies to commercialisation. Along this journey, some of key professionals required to shepherd a new medicine through these hurdles will be introduced, including those engaged with funders to support the long journey, those with expertise in talking with regulators to align program activities with the eventual requirements for registration, those who can help selecting a regulatory pathway best suited to the asset and indication, those who can develop and oversee manufacturing at the quality required for nonclinical and eventual scale required for clinical studies, and the teams of those required in the designing and conducting nonclinical safety studies in a way that satisfies multiple global regulators while minimising the unnecessary use of animals. These activities need to work in sync with the design and operation of the huge team response for the clinical programs so that the desired scientific outcomes can be achieved while ensuring patient safety and operational efficiency. Finally, the importance of building a program and network to eventually partner the product to support registration and global patient access will be discussed. This presentation will detail some of the tools and strategies needed to manage the cross-disciplinary teams required to minimize the time, cost and risk of translating new biomedical discoveries into new medicines in a biotech company. DiMasi JA, Grabowski HG, Hansen RA. Innovation in the pharmaceutical industry: new estimates of R&D costs. Journal of Health Economics 2016;47:20-33.

203. Application of organoids to accelerate drug discovery into the patient

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The drug discovery process is complex, time-consuming, and expensive, and high risk due to our limited understanding of many diseases. Because of the complexity of human diseases, our models, which mostly rely on 2D cell lines from many species and animal models, are frequently oversimplified. Unfortunately, these models frequently fail to completely depict the complexities of diseases, despite the fact that their outcomes are crucial in decision making around drug candidate progression.1 In recent years, the use of organoids has emerged as a cutting-edge approach in preclinical research, garnering significant interest in drug discovery. 2 Organoids, three-dimensional cellular models derived from human cells, closely mimic the shape and function of real human organs, offering unparalleled physiological relevance. Moreover, their creation from patient-specific cells opens avenues for personalised medicine, enabling the testing of drug candidates tailored to individual genetic profiles. However, organoids cannot completely replicate the complexity of entire organs, potentially leading to an incomplete understanding of drug effects. Despite these challenges, the use of organoids in drug discovery shows immense potential. They provide a transformative platform that combines precision and human relevance, significantly enhancing our ability to develop safer and more effective drugs. This presentation will provide an overview of the pros and cons associated with the application of organoids in the drug discovery process.

1. Scannell et al (2022) Nature Reviews Drug Discovery, 21:915-931. 2. Jeya Vandana et al (2023) Cell Stem Cell, 30:571-591.

204. Discovering and translating drugs to treat coronary artery disease beyond standard modifiable risk factors

Prof Gemma Figtree, The University of Sydney

Cardiovascular disease remains the leading cause of death globally. Despite the tremendous progress in heart attack prevention seen through the screening and treatment of high cholesterol and blood pressure, between 10-27% of first time heart attack patients have no such modifiable risk factors. Furthermore, some patients are observed to rampage on with atherosclerosis progression despite compliance with best practice LDL-lowering and BP-lowering treatments. Such clinical observations highlight the urgent unmet need for drugs targeting new factors in atherosclerosis susceptibility. The clinical translational pipeline for new coronary artery disease drugs is a substantial disincentive for investment in discovery. Preclinical models poorly reflect the human disease, and even our closest relatives the chimpanzees who have a 50% higher cholesterol than us, do not suffer spontaneous coronary artery disease and plaque rupture with heart attack, other than under extreme experimental conditions. The need to perform clinical trials in humans has been challenging related to the lack of valid surrogates until now, and the need for large randomised clinical trials powered for heart attack and death. A number of recent strategies have been developed to address the challenges of translating new drugs for human CAD, including patient-derived cellular and organoid models; novel animal models reproducing plaque instability and rupture; and new clinical trial platforms utilising advanced non-invasive imaging of non-calcified coronary plaque. Early engagement with regulatory authorities, as well as consumers, and clinicians is also critical for a shift in the dial.

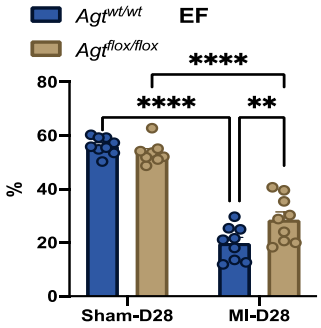
205. Lessons from the lung - current and novel approaches for treating fibrosis

A/Prof Jane Bourke, Monash University

Fibrosis is a hallmark feature of interstitial lung diseases, including idiopathic pulmonary fibrosis (IPF), with the development of fibrotic foci in the parenchyma reducing gas exchange and causing progressive loss of lung function. Oral pirfenidone and nintedanib are current standard-of-care treatments that slow progression but do not reverse established disease. While the recent development of an inhaled formulation of pirfenidone offers promise in limiting its common adverse effects, there is still an urgent need for novel antifibrotic drugs to improve the prognosis for these patients. While transforming growth factor β1 is considered the main driver of fibrosis, emerging evidence supports a role for the pulmonary renin-angiotensin system in the pathogenesis of IPF. The angiotensin type 2 receptor (AT2R) is reported to play a protective role , opposing pro-fibrotic effects on angiotensin II mediated via the AT1R. Activation of AT2R represents a potential novel anti-fibrotic treatment strategy for IPF, and the development and assessment of highly selective AT2R agonists is an area of active investigation. This presentation will outline our current understanding of IPF pathophysiology, extending beyond the well-characterised contributions of TGFβ1 to include potential roles for angiotensin II and related peptides. Preclinical assessment of currently available and novel AT2R agonists will be described, with comparisons to current treatments in human IPF cells, human precision-cut lung slices and bleomycin mouse model of IPF. A particular focus will be on compound 21, an AT2R agonist currently in clinical trials for IPF.

206. Inactivation of the local cardiac renin angiotensin system improves cardiac performance after myocardial infarction

Zunhan Lu, Yusuke Yoshikawa, Monique Lowes, Melisa Reichelt, Walter Thomas. School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia.



Introduction. The renin-angiotensin system (RAS) regulates blood pressure via angiotensin II (Ang II), which is generated in blood from angiotensinogen (AGT). In addition to the blood borne RAS, a local cardiac RAS has been identified that is activated following heart injury. Based on RNAseq data from isolated cardiac cells (1), AGT is primarily expressed from cardiomyocytes.

Aims. We aimed to examine the role of the local cardiac RAS in the remodelling process post-myocardial infarction (MI) by specifically deleting AGT from adult cardiomyocytes to prevent local activation of the RAS.

Methods. Four weeks prior to myocardial infarction $AGT^{wt/wt}$ and $AGT^{fl/fl}$ mice were injected with adeno-associated virus (AAV9), which drives the expression of Cre enzyme specifically in cardiomyocytes. The expression of transgenes and AGT deletion were confirmed by RT-qPCR; fibrosis was analysed by histological staining and cardiac function post MI was assessed by echocardiography.

Results. The AAV-Cre approach successfully deleted AGT in cardiomyocytes. This AGT knockdown reduced MI-induced inflammatory, hypertrophic, and fibrotic responses at 7 days after MI. At 28 days after MI, control mice ($AGT^{wt/wt}$) showed profound impairment of cardiac output, stroke volume and ejection fraction (see figure), whereas AGT deleted $AGT^{fl/fl}$ mice showed significantly improved systolic cardiac function.

Discussion. These results indicate a functional, local cardiac RAS, which is active following myocardial infarction and contributes to the fibrosis and functional impairment associated with cardiac damage/repair. The findings of this study provide fundamental insights into the contribution of the local RAS in the setting of cardiac pathology and may have clinical relevance when considering local versus systemic RAS inhibition.

1.Quaife-Ryan Gregory A, Sim Choon B, Ziemann M, Kaspi A, Rafehi H, Ramialison M, et al. Multicellular Transcriptional Analysis of Mammalian Heart Regeneration. Circulation. 2017;136(12):1123-39.

207. Targeting the AT2 receptor as an effective means of treating fibrosis

Dr Yan Wang, Monash University

Pathological fibrosis (scarring) results from the excessive accumulation of extracellular matrix (ECM) proteins, primarily collagen, and is a hallmark of organ dysfunction. Angiotensin (Ang) type-2 receptor (AT2R) stimulation is recognised as a protective strategy that counter-balances overactivity of the reninangiotensin system (RAS) at various levels. We have recently synthesised >100 peptides that are highly selective to the AT2R. In this presentation, I will discuss the anti-fibrotic and anti-inflammatory properties of some of these novel ligands and the potential signalling mechanisms involved.

208. An update on how the RAAS can be targeted to treat liver fibrosis

Chandana B Herath1 and Peter W Angus2 1Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria, Australia. 2Department of Gastroenterology and Hepatology, Austin Health, Heidelberg, Victoria, Australia.

Liver fibrosis and its sequelae of liver cirrhosis and liver cancer is now one of the world's leading causes of chronic illness and death. However, at present, there is no specific medical treatment for this condition. There is considerable experimental evidence that the renin angiotensin aldosterone system (RAAS) plays a central role in liver fibrogenesis. Whilst the classical RAAS, comprising angiotensin converting enzyme (ACE), angiotensin II (Ang II) and its putative receptor Ang II type 1 receptor (AT1R) is profibrogenic, the alternate RAAS, also known as the protective RAAS, comprising angiotensin converting enzyme 2 (ACE2), angiotensin-(1-7) (Ang-(1-7)) and its putative receptor Mas (MasR) opposes many of the deleterious effects of the classical RAAS. The primary mechanism for this is ACE2 mediated degradation of profibrotic Ang II peptide to the anti-fibrotic Ang-(1-7) peptide. Thus, the balance between the activity of the two axes of the RAAS dictates its

overall effect on organ scarring. Since the protective RAAS is a potential anti-fibrotic target, we investigated the therapeutic effect of ACE2 in liver fibrosis. In a series of studies using mouse and rat models of liver disease, ACE2 gene was delivered using a safe and liverspecific adeno-associated viral (ACE2-AAV) vector via intraperitoneal (i.p.) route to over-express ACE2 in the liver. ACE2 gene therapy greatly increased liver ACE2 gene expression compared to a control human serum albumin (HSAAAV) vector and lasted for at least 6 months after a single i.p. injection. ACE2 therapy improved hepatocellular damage as reflected by significantly reduced liver enzyme profiles compared to control vector injected animals. ACE2 therapy significantly downregulated the expression of proinflammatory and profibrotic cytokines, NOX enzyme subunits and generation of reactive oxygen species compared to the controls. These changes were associated with a major reduction in the activation of hepatic stellate cells, the key cell type that secretes extracellular matrix proteins in response to tissue injury, leading to a significant reduction in matrix component collagen secretion. The profound reduction in proinflammatory and profibrotic cytokines in ACE2 treated animals and associated major reduction in liver fibrosis has been demonstrated in animal models of liver disease induced by a variety of insults including high-fat high-cholesterol diet, methionine and choline deficient diet, biliary obstruction and toxic agents. Mechanistically, these beneficial changes during ACE2 therapy were associated with increased hepatic Ang-(1-7) peptide levels and degradation of hepatic Ang II levels. In conclusion, these findings suggest that liver-specific ACE2 gene therapy has a profound beneficial effect on liver fibrosis. We therefore conclude that liver-specific ACE2 upregulation has potential as a therapy for patients with liver fibrosis.

209. Advances in toxicology inform health risk assessment: The role of ACTRA in Australasia

Brian G Priestly, School of Public Health & Preventive Medicine, Monash Univ, Melbourne, VIC, Australia.

In introducing this symposium on ‘Transformations in toxicology’ I would like to take the opportunity to introduce the Australasian College of Toxicology and Risk Assessment (ACTRA) and to indicate how ACTRA has contributed, and will continue to contribute, to the development of the discipline of toxicology in Australasia. ACTRA was formed in 2006 following a 2-day symposium highlighting achievements and challenges in environmental health risk assessment in Australasia. ACTRA received seeding fund and ongoing support from several partners in the government’s enHealth Council. In addition to establishing a peer-reviewed register of registrants and fellows, to provide a form of professional accreditation, ACTRA has supported continuing education programs through Annual Scientific Meetings (ASM), workshops and webinars. The ASMs offer an opportunity for members (and non-members) to make presentations on their work. Themed sessions on selected topics generally include high-quality invited presentations from national and international speakers. A selection of the topics covered in themed ASMs, workshops and webinars will be shown and demonstrate the scope of ACTRA’s activities. ACTRA is affiliated with the International Union of Toxicologists (IUTOX) and our former President (Peter Di Marco) was the first Australian elected as President of IUTOX (2019-22). ACTRA recognises the changing nature of toxicological analysis and the role it will play in the future development of health risk assessment functions across regulatory and environmental formats. A focus on New Assessment Methods (NAMs) and the role they will play in the future of toxicology has been a consistent feature of ACTRA’s programs and forms the basis for what will be presented in this Symposium.

210. Toxicokinetic solutions for dynamic environmental problems

Authors: Antti T. Mikkonena,b; Jennifer Martinb , Richard N. Uptona , Mark Patrick. Taylorb , Michael S. Robertsa,d, Lorraine Mackenziea,e a)University of South Australia, Clinical and Health Sciences, Adelaide, South Australia, Australia; b)Environmental Protection Authority Victoria, Australia; d)University of Queensland, Frazer Institute, Queensland, Australia; e)Basil Hetzel Institute for Translational Health Research, The Queen Elizabeth Hospital, Woodville South, South Australia, Australia Environmental risk assessment often relies on static estimates and steady state assumptions.

This presentation focuses on a case when these assumptions do not apply and a new dynamic approach was developed. The presence of per- and polyfluoroalkyl substances (PFAS) in the food chain presents an ongoing challenge for risk assessment and risk management. While concentrations of PFAS in food in Australia is generally low, consumption of food is one of the primary PFAS exposure routes for the general population and particularly for local communities near contaminated sites. PFAS occurrence has been reported in a range of agricultural commodities including cattle, in Europe, Australia and the United States. Exposure assessment and management for consumers are complicated by the lack of validated modelling approaches to estimate PFAS bioaccumulation in cattle. Previous studies have shown that bioaccumulation in cattle is influenced by environmental, spatial, weather and temporal factors that necessitate a dynamic modelling approach. This work presents an integrated exposure and population toxicokinetic (PopTK) modelling approach that estimates PFAS bodyburden in cattle over time based on daily (variable) exposure. This approach was developed as a practical tool to manage livestock risks on PFAS impacted farms and to aid with prioritisation of remedial actions for sites.

211. Use and Assessment of New Approach Methods by the Australian Pesticides and Veterinary Medicines Authority

Rhian B Cope1 and Selma Kaasinen2 1. Principal Toxicologist, APMVA 2. Director, Health Assessment Team

APVMA (APVMA) regulates agricultural and veterinary chemicals and related products in Australia to manage the risks of pests and diseases to Australian community as well as to protect the safety of people, animals and environment. The APVMA regulates products up to the point of sale: control of use is regulated by the States and Territories. New application to the APVMA go through a robust scientific evaluation and approval process before it can be accepted, and the new active/product is entered to the APVMA register. The data requirements are tailored based on the type of application (i.e., new chemical, new product, permit) and possible concerns (i.e., related to chemistry, environment, health, residues, safety and efficacy). APVMA accepts data generated based on international (i.e., OECD/VICH guidance) or based on other comparable test guidance documents (such as national data requirements). In addition, APVMA has a policy of accepting studies providing bridging information or weight of evidence. For instance, APVMA has accepted human relevance studies, i.e., mode of action studies on carcinogenicity for years. Similarly, studies based on the New Assessment Methods (i.e., in vitro skin and eye irritation, DASS, in chemico studies) are evaluated and considered by their suitability providing weight of evidence. The APVMA focuses on an outcome-based assessment process, ensuring satisfaction with safety criteria, and looks to utilize available assessment methodologies as part of contemporary assessment.

212. Strengths and weaknesses of PBPK modelling in a regulatory context

Michael S. Roberts1,2 1 Frazer Institute, Faculty of Medicine, University of Queensland, Brisbane, Australia 2Clinical & Health Sciences, University of South Australia, Basil Hetzel Institute for Translational Health Research, Adelaide, Australia

Physiologically based pharmacokinetic modelling (PBPK) is commonly used to predict exposures of drugs and environmental toxins when there is limited human data to fully define the internal dose in a target organ. Here, we first show how PBPK modelling, development and validation criteria can be used in toxicological risk assessments. We then consider PBPK advantages and weaknesses in a regulatory context. Finally, we consider a number of examples where PBPK has been used in risk assessment for various actual or potential toxins, where we show how PBPK models can be applied in predicting human equivalent dose values from animal no and low adverse effect levels (i.e., NOAEL, LOAEL) using interspecies, route to route and high dose to low dose extrapolation.

213. What can DNA tell us about the human settlement of the Pacific and why does it matter?

Prof Lisa Matisoo-Smith, University of Otago

DNA technology has rapidly developed in the last decade, allowing for the generation of a large amount of DNA data from both ancient and living peoples, plants and animals from across the globe, including the Pacific region. These new data and their interpretation are providing new insights into our understanding of the settlement of the Pacific and challenging many long held views. Perhaps more importantly, when combined with data and questions from other fields, our understanding of genetic variation and settlement history can be used to better understand and hopefully treat some of the health issues that disproportionately impact Pacific peoples. Finally, I hope to demonstrate how collaborative research agendas, particularly involving communities, can identify new questions of interest and interpretations that will benefit all involved.

214. Oncology Pharmacogenomics and Aboriginal Australians

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The contribution of precision medicine to the Australian health care system is increasing; however, it is crucial that it be inclusive of the diversity of the Australian population, uniquely that of Indigenous Australians. Several oncology drugs are now being or about to be tested for pharmacogenetic variants that are associated with severe and potentially life-threatening toxicities. These are the fluorouracils/capecitabine (DPYD), mercaptopurine/thioguanine (TPMT/NUDT15) and irinotecan (UGT1A1). Although the frequencies of these gene variants are well known in many populations with striking biogeographic regional specificities, there is nothing known about Indigenous Australians. We aimed to compare the frequencies of clinically important DPYD, TPMT, NUDT15 and UGT1A1 variants in a cohort of Aboriginal Australians (AA) with a European Australian (EU) cohort investigated at the same time. Following approval from the Aboriginal Health Research Ethics Committee (a subcommittee of the Aboriginal Health Council of South Australia) and the University of Adelaide Human Research Ethics Committee, we collected a saliva sample from 149 Aboriginal (AA) and 263 European (EA), Australians and using the DMET array tested for: 18 DPYD, 6 TPMT, 32 UGT1A1 polymorphisms and for NUDT15 alleles we used targeted next generation sequencing. For DPYD, the only nonfunctional *2A allele frequency was not different (0.3-0.6%) between the two groups; for TPMT the nonfunctional *3A frequency was significantly reduced from 4.6% to 1.4% (OR:0.29) and the combination IM/PM phenotype was significantly lower in AA (OR=0.32); for NUDT15 the nonfunctional *3 allele was not different (AA:1.1%, EA:0.4%) and for UGT1A1 the decreased function *6 allele was significantly higher in AA (3.7% vs 0.19%) whereas the *28 allele was lower (AA: 0.20% vs EA 0.32%) but there was no difference in overall phenotype frequencies. In summary, for the 4 genes that could be tested for toxicity in oncology drugs, Aboriginal Australians do not appear to have frequencies of nonfunction or reduced function alleles which are

substantially different to European Australians. Whether there are rare variant alleles that affect function and whether Aboriginal Australians are more prone to the toxicity of oncology drugs at standard doses is unknown.

215. An update on pharmacogenetics relevant for New Zealand Māori and Pacific peoples and the challenges for equitable access to "precision medicine".

Dr Nuala Helsby, University of Auckland

Allele frequencies in pharmacogenes which influence drug disposition and response can vary substantially across global populations. Historically pharmacogenomics research has focused on people from Europe and East Asia, unfortunately knowledge of pharmacogenetic variation in small nations and indigenous peoples is vastly under-represented. Lack of inclusion of people of nonEuropean ancestry can underestimate drug-gene associations which involve novel variants predominantly observed in other ethno-geographic populations. This results in poor generalisability of findings, which could translate into healthcare disparities. This talk will survey what is currently known about pharmacogenetic variation in people from across Polynesia. It will also highlight the importance of Māori cultural values of taonga and kaitiakitanga and how respectful initiatives to ensure community consent, support and engagement can build appropriate capacity to produce collective benefit.

216. β_2-Adrenoceptor-Mediated Invasion in Triple Negative Breast Cancer is Driven by HOXC12

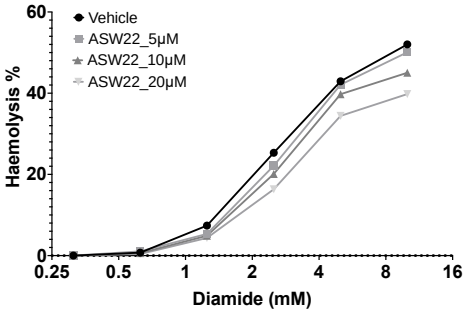
Terrance Lam[1], Bailey Cardwell[1], Bonan Liu[1], Alastair C Keen[1], Aeson Chang[1], Erica K Sloan[1], Michelle L Halls[1].
[1]Drug Discovery Biology Theme, Monash Inst Pharm Sci, Monash University, Parkville, VIC, Australia

Introduction. Noradrenaline released from sympathetic nerves during chronic stress accelerates cancer metastasis by activating β_2-adrenoceptors (β_2ARs) on tumour cells to promote invasion. We previously identified that the β_2AR drives invasion via a cAMP/calcium (Ca^{2+}) feedforward loop in the highly metastatic triple negative breast cancer (TNBC) cell line MDA-MB-231^{HM} (Pon et al, 2016). However, the commonality of this mechanism remains unknown.
Aims. To determine whether the β_2AR-cAMP-Ca^{2+}-invasion pathway is a common feature of TNBC.
Methods. Formoterol activation of the endogenous β_2AR was assessed in 11 TNBC cells. Interplay between cAMP and Ca^{2+} signalling was determined by measuring cAMP or Ca^{2+} in the presence of a Ca^{2+} chelator (BAPTA-AM) or an adenylyl cyclase inhibitor (2',3'-dideoxyadenosine, ddA), respectively. Invasion was assessed using microscopy. Principle component analysis (PCA) of transcriptomic and proteomic data was conducted to identify differentially expressed genes/proteins between cells that possess the feedforward loop compared to those that do not.
Results. There was no effect of formoterol on cAMP or Ca^{2+} in two TNBC cells (HCC1937, MDA-MB-453). Formoterol increased cAMP and Ca^{2+} in six of the remaining nine TNBC cells: HCC38 (pEC_{50} cAMP 8.58±0.44, Ca^{2+} 7.90±0.22; n=6-8), HCC1143 (pEC_{50} cAMP 9.88±0.33, Ca^{2+} 9.70±0.25; n=4-6), HCC1806 (pEC_{50} cAMP 8.88±0.48, Ca^{2+} 8.98±0.38; n=4-6), BT549 (pEC_{50} cAMP 9.48±0.28, Ca^{2+} 9.65±0.70; n=4-5), MDA-MB-468 (pEC_{50} cAMP 9.08±0.40, Ca^{2+} 9.07±0.17; n=4-6), HCC1395 (pEC_{50} cAMP 8.26±0.31, Ca^{2+} 7.73±0.58; n=4). BAPTA-AM and ddA inhibited cAMP and Ca^{2+}, respectively, suggesting that a cAMP/Ca^{2+} feedforward loop exists in these cells. Activation of the cAMP/Ca^{2+} feedforward loop correlated with accelerated invasion following β_2AR stimulation. PCA identified higher expression of the *HOXC12* transcription factor in cells with the feedforward loop. CRISPR knockdown of HOXC12 uncoupled the β_2AR from the cAMP-Ca^{2+}-invasion pathway.
Discussion. High expression of HOXC12 drives the β_2AR-cAMP-Ca^{2+}-invasion pathway in TNBC cells.

217. Small molecule activation of glucose-6-phosphate dehydrogenase (G6PD)

Joshua Storm Caley[1], Ashley S.A. Wong[1], Claire Weekley[2], David A. Sinclair[3], Michael Parker[2], Kate Michie[1], David Jacques[1] & Lindsay E. Wu[1]

School of Biomedical Sciences, UNSW[1], Sydney, NSW, Australia; Bio21 Institute, University of Melbourne[2], Melbourne, VIC, Australia; Blavatnik Institute, Harvard Medical School[3], Boston, MA, United States



Introduction. Glucose-6-phosphate dehydrogenase (G6PD) serves as the rate-limiting enzyme in the pentose phosphate pathway, which regenerates glutathione to deal with reactive oxygen species (ROS), and provides NADPH and nucleotides to fuel cell growth. G6PD overexpression extends lifespan in model organisms, and its mutation in humans causes Favism, which affects 400 million people, and is characterised by the risk of haemolytic anaemia caused by ingestion of foods that promote ROS formation.

Aims. We aimed to identify small molecule activators that improved G6PD enzyme activity, and their impacts on human erythrocyte function, which are highly susceptible to G6PD deficiency.

Methods. A small molecule drug screen identified a series of G6PD activators. In vitro enzyme activity assays and NanoDSF studies were used to test the impact of these compounds on G6PD protein stability, while ex vivo experiments on human erythrocytes were used as a functional assay for the prevention of ROS induced haemolysis.

Results. We identified three compounds that improved G6PD enzyme activity; however, rather than being true allosteric activators, they act by maintaining protein stability. These compounds could reduce haemolysis in human erythrocytes.

Discussion. G6PD deficiency is the most common human enzyme mutation, affecting over 400 million people. Given its role in cellular metabolism and protection, declining G6PD activity with age might contribute to biological ageing. We have identified new compounds that maintain G6PD enzyme activity, and show efficacy against ROS induced haemolysis in human blood.

218. Physicochemical graph neural network for learning protein-ligand interaction fingerprints from sequence data

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In drug discovery, determining the binding affinity and functional effects of small-molecule ligands on proteins is crucial. Computational methods have been developed to predict these protein-ligand interaction properties, enabling efficient exploration of chemical spaces. However, the accuracy of computation methods often depends on high-resolution protein structures, and these methods can fall short in predicting functional effects. We introduce PSICO (PhySICOchemical graph neural network), a method designed to adhere to physicochemical principles while decoding protein-ligand interaction fingerprints directly from sequence data. Unlike previous sequence-based models, PSICO incorporates physicochemical constraints to achieve both accuracy and interpretability. Our results show that PSICO matches, and even surpasses, structure- and complex-based methods when trained on the same protein-ligand pairs without using structural and complex-based information. Additionally, PSICO excels in predicting the functional impact of small molecules on protein targets. In a proof-of-concept study, we applied PSICO to screen a library of 30,282-compound for adenosine A_1 receptor (A_1R) agonists. Impressively, PSICO ranked the novel A_1R agonist identified from a high-throughput screening campaign of this library, according to pharmacological evaluation, within the top 3. Moreover, PSICO's fingerprints are interpretable, identifying protein residues and ligand atoms involved in interactions. We anticipate PSICO will revolutionaize virtual ligand screening and deepen our understanding of protein-ligand interactions.

219. Exploring Spexin-1 and Predicted Spliceoforms: Insights into GPR161 and GAL_2R Signalling

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Introduction. In recent years, the use of *in silico* methods for the prediction of endogenous peptide ligands and their GPCRs has led to the identification of several novel pairings[1]. In one such study, the neuropeptide spexin-1 was proposed to be a potential ligand for the orphan GPCR, GPR161[1]. This neuropeptide has been shown in literature to bind galanin 2 (GAL_2) and 3 (GAL_3) receptors and has a physiological function that is seemingly reciprocal to their endogenous agonist, galanin[2]. Moreover, the precursor peptide for spexin-1 reveals several dibasic cleavage sites, indicating the possibility of multiple mature spexin spliceoforms.
Aims. To characterise the signalling profiles of spexin-1 and predicted spliceoforms at GPR161 and GAL_2.
Methods. Proximal interactions between GPCR and downstream effector molecules such as G alpha (Gα) proteins and β-arrestins, and downstream extracellular signal-regulated kinases (ERK) activation were quantified using bioluminescence resonance energy transfer (BRET) assays while changes in distal second messenger levels were measured using the cyclic AMP response element (CRE) or serum response element (SRE) reporter gene assays, upon ligand treatment. Appropriate positive controls were established for all assay systems in each individual experiment.
Results. Spexin-1 and its spliceoforms did not activate GPR161 through canonical G protein-dependent and -independent signalling pathways. Spexin-1 stimulated GAL_2, not only through G protein engagement, but also via β-arrestin2 recruitment. Several of the predicted spexin spliceoforms were found to be biologically active and stimulated GAL_2 in a G protein-dependent signalling assay.
Discussion. As the galanin receptor signalling axis is important for many physiological processes in the human body such as feeding, mood regulation and more, identification of novel agonists for these receptors may help uncover potential biased signalling mechanisms and ultimately improve therapeutics. Future experiments will aim to uncover differences in binding of spexin spliceoforms at GAL_2.
[1] Foster SR et al. (2019) Cell 179:895-908.e821
[2] Lv SY et al. (2019) Front Pharmacology 10:457

220. A novel single-cell fluorescence microscopy-based analysis for the detection of biomarkers of cellular senescence

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Introduction. Cellular senescence is a complex state of irreversible cell cycle arrest with a secretory phenotype associated with age-related diseases. Senescence-associated beta galactosidase (SA-β gal) is one of the hallmark biomarkers of senescent cell detection along with the increased P53 and P16 expression, and cell and nucleus areas. Traditional method of SA-β gal determination employs cytochemical or histochemical staining and manual counting of positively stained cells. This method, however, has multiple disadvantages. In addition to its laborious procedure, these approaches often assess a population of cells as a whole. As a result, given the considerable heterogeneity among cells of a population in the level of different senescence markers, these analyses do not show a robust induction of senescence. This is particularly a disadvantage for developing drugs against senescence.
Aim. To develop a novel method of single-cell detection and analysis of senescence markers.
Methods. To establish an accelerated model of senescence, human-derived fibroblasts were treated with the chemotherapeutic agent, Mitomycin C (MMC) (50-600 nM), or vehicle for 72 h, and further cultured in normal media for five days. IN Cell Analyzer 2200 microscope was used to detect SA-β gal (stained using a commercial fluorescent enzymatic kit), and P21 and P16 proteins (stained by antibodies). IN Carta Image Analysis Software was used to quantify senescence markers. An “induction threshold” value was set by determining the value below which 90% of values in the control no-MMC group lie. Accordingly, percent of values above threshold was assigned for each group.
Results. Assessment of fluorescent intensity of SA-β gal, showed an around 2-fold increase in the average values of all cells with MMC treatment. We next plotted a histogram of values binned based on different SA-β gal fluorescence levels in cells. This histogram clearly indicated the considerable heterogeneity in SA-β gal levels. Using our “induction threshold” method, we showed an around 19-fold increase in SA-β gal levels in MMC-treated groups compared to the control. Similar trend of results was found in other biomarkers of cellular senescence including P53 and P16 expression, and cell and nucleus areas.
Discussion. We developed a novel analysis method of cellular senescence biomarkers at single-cell level. This method helps in identifying sub-populations of senescent cells and provides a robust platform for senotherapeutic discovery.

221. Characterisation of the pro-atherosclerotic orphan G protein-coupled receptor, GPR146

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Introduction. GPR146 is an orphan G protein-coupled receptor that has a convincing pro-atherosclerotic role through upregulation of the cholesterol biosynthesis pathway. Inhibition of this receptor may be particularly useful with treatment-refractory familial hypercholesterolaemia. However, the molecular pharmacology of this receptor remains understudied. Proinsulin C-peptide and foetal bovine serum (FBS) are proposed activators of GPR146, although the pairing with C-peptide has not yet been reproduced by an independent research group and the active component in FBS has not yet been identified.
Aims. The aim of this study was to validate previously proposed ligands for GPR146.
Methods. C-peptide and FBS were tested using the following assays: reporter gene assays to investigate Gαs, Gαi/o, Gαq/11, and Gα12/13 signalling; a NanoBiT assay for β-arrestin recruitment; and Western blot or a BRET1-based biosensor for ERK1/2 phosphorylation (pERK1/2). A panel of 58 human sera was screened at GPR146 using Western blot probed for pERK1/2; the threshold for "hit" selection was set at ±2xSD. Human sera identified as "hits" were then further characterised using G protein- and arrestin-deficient HEK293A cells.
Results. Neither C-peptide nor FBS activated GPR146 in any assays tested (n=5); assay validity was confirmed by multiple positive controls. An overall increase in pERK1/2 was observed in response to human serum in GPR146-expressing cells compared to cells not expressing GPR146 ($P<0.0001$, paired t-test). 47/58 human serum samples elevated pERK1/2, with 5 surpassing the upper hit threshold indicating activation of GPR146.
Discussion. In this study, previously proposed ligands for GPR146 were not reproduced, indicating that C-peptide is not, and FBS does not contain, the endogenous ligand for GPR146. Instead, human serum was identified as an activator of GPR146. Future studies with human serum may identify the endogenous ligand for GPR146.

Yu et al. 2019. Cell. 179(6):1276-1288.e14.
Yosten et al. 2013. J Endocrinol. 11;218(2):B1-8.

222. Extracellular vesicles as a liquid biopsy to predict drug exposure and response.

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Introduction. The capacity to define target engagement and variability in exposure to new drug candidates are key elements of early clinical drug development. Historically this has been challenging to achieve at a molecular level. Extracellular vehicles (EVs) are small, membrane-encapsulated particles that are released into biofluids including blood by virtually all cells. EVs contain an array of protein and nucleic acid cargo. The capacity of EV encapsulated cargo to reflect the state of their tissue of origin has driven the prominent rise of EVs as a liquid biopsy.
Aims. The aim of this research program has been to establish the function of proteins derived from circulating liver and intestine specific extracellular vesicles as markers of variability in drug exposure and target engagement.
Methods. EVs were isolated from the extracellular matrix of human liver and intestinal tissue using our established size exclusion chromatography (SEC) protocol. Tissue specific EVs are isolated from plasma using our two-step protocol that involves the initial isolation of all EVs (global EVs) using SEC, followed by enrichment of tissue specific EVs by immunoprecipitation using antibodies against organ specific proteins. Untargeted liquid chromatography-mass spectrometry (LC-MS) analyses were performed to identify proteins in matched tissue and EVs. Targeted LC-MS assays were deployed to quantify absolute abundances for a panel of key drug metabolising enzymes and drug targets.
Results. Untargeted LCMS analyses detected 2892 proteins in liver EVs (LEV) and 2673 in liver homogenate (LH). Of the 2673 proteins detected in LH, 1547 (58%) were also detected in LEV. Bioinformatic analyses demonstrated comparable representation of proteins in terms of biological functions and cellular compartments. Targeted analyses demonstrated robust concordance ($r > 0.5$) between expression in LEV and LH for a panel of 20 drug metabolising enzymes and protein targets for medicines used in the treatment of cancer and liver disease. Eighteen out of 20 protein targets quantified in tissue derived EVs were quantifiable in circulating tissue specific EVs.
Discussion. These data demonstrate the feasibility of isolating tissue specific EVs from plasma and quantifying the tissue specific abundance of key proteins that define drug exposure and response. This project has further established a dedicated workflow that can be applied in early clinical trials to provide the first direct clinical assessment of key pharmacokinetic and pharmacodynamic characteristics for new drug candidates.

223. Dichloroacetate decreases dihydropyrimidine dehydrogenase activity in cancer patients: translation from mouse pharmacogenomics.

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Introduction: Dichloroacetate (DCA) is a pyruvate dehydrogenase kinase inhibitor with anti-cancer activity, reversing the metabolic Warburg effect. At clinical doses, DCA alone primarily inhibits proliferation, but enhances chemotherapy cytotoxicity and thus is likely to be used in combinations. Previous studies have shown that DCA can alter paracetamol and cisplatin toxicity in mice through changes in xenobiotic metabolism genes. *Dypd* encodes dihydropyrimidine dehydrogenase (DPD), the primary liver enzyme responsible for de-activating 5-fluorouracil / capecitabine (5-FU). Deficiency in DPD contributes to more severe 5-FU toxicity in approximately 20% of 5-FU patients.
Aims: We have catalogued changes in RNA encoding drug metabolizing enzymes in DCA-treated mice, to identify other potential drug interactions due to altered drug metabolism. We also provide evidence from the DiCAM clinical trial of DCA in multiple myeloma patients (Tian et al 2019) that similar changes can occur in humans at clinical doses.
Methods: High throughput sequencing of RNA from liver of untreated mice and mice treated with DCA (250 mg/kg i.p. for 5 days) was performed and differentially expressed genes were examined for members of candidate drug metabolism pathways. DPD activity in DiCAM patients was assessed by measurement of the dihydrouracil / uracil ratio (UH_2/U) by HPLC-UV in paired serum or plasma samples from before and after DCA treatment.
Results: Significant changes were found in 335 genes ($p<0.001$, fold-change >1.5), including 8 cytochrome P450s, 4 glutathione transferases, 3 sulfotransferases, 2 glucoronidases. A 28% reduction ($p=0.0008$) in mRNA levels of *Dpyd* was also found. Analysis of DPD activity in 4 cancer patients taking DCA, found a reduction in UH_2/U in all 4 patients ($p=0.02$, ratio paired t-test), putting these patients at risk of severe toxicity from 5-FU.
Discussion: Screening mouse liver gene expression changes with DCA therapy has been successful in identifying a potential drug interaction in humans. Trials involving DCA in combination with 5-FU should monitor toxicity closely.

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224. Hepatic Protein Signatures of Chronic Polypharmacy, Monotherapy, and Deprescribing in Aged Mice

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Introduction. Polypharmacy is common in old age and is associated with adverse geriatric outcomes. Deprescribing medications may alleviate some outcomes. The liver is a key metabolic organ and is affected by drugs and ageing.
Aims. In aged mice, to investigate (i) the effect of chronic polypharmacy, monotherapy, and deprescribing on the hepatic proteome; and (ii) the relationship between the hepatic proteome and geriatric outcomes.
Methods. Healthy C57BL/6J mice aged 12 months commenced either (i) control, (ii) chronic monotherapy (oxybutynin, oxycodone, citalopram, simvastatin, or metoprolol at therapeutic doses), or (iii) chronic polypharmacy (all 5 monotherapies). At 21 months, treated mice were re-randomised to continue or deprescribe medications. Livers and serum were collected at age 26 months. Proteomics analysis was performed by data-independent acquisition (DIA) using a Q-Exactive (Hfx) orbitrap mass spectrometer (n=5-8/group). Serum drug levels were measured. Differential expression, pathway, and network analyses were applied.
Results. Compared to control, several proteins were differentially expressed with monotherapy (oxybutynin: 26, oxycodone: 38, citalopram: 74, simvastatin: 36, metoprolol: 55; $p<0.05$, fold-change threshold: ±1.50). With polypharmacy, 243 proteins were differentially expressed, of which 178 (73%) were unique to polypharmacy. The polypharmacy proteome signature is characterised by proteins involved in drug metabolism, immune function, and cholesterol biosynthesis. Serum levels of citalopram, noroxycodone, and metoprolol were 2-fold higher in polypharmacy than monotherapy ($p<0.05$). Complete deprescribing reverses up to 50% of changes in the hepatic proteome. Frailty was negatively correlated with lipid metabolic proteins (r=-0.27 to -0.28; $p<0.05$). Distance travelled (r=0.22, $p<0.05$) and rotarod endurance time (r=0.42, $p<0.05$) correlated with amino acid metabolic proteins.
Discussion. Polypharmacy altered expression of hepatic proteins, to a greater extent than all monotherapy, with partial reversal after deprescribing. Drug metabolism, immune function and cholesterol biosynthesis may drive effects of this polypharmacy regimen. Amino acid and lipid metabolism proteins were associated with adverse geriatric outcomes.

225. Clozapine-Induced Cardiotoxicity: Investigating reactive species associated with metabolite cycling.

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Introduction. Clozapine is an antipsychotic, limited in its prescription by adverse drug reactions, including cardiotoxicity. Metabolism of clozapine is implicated in clozapine toxicities, yet whilst one of its circulating metabolites (*N*-desmethylclozapine) is routinely studied the other (clozapine-*N*-oxide) remains under-investigated.
Aims. To identify if cytochrome P450 (CYP) isoforms, including those expressed in the heart, catalyse oxidation and reduction of clozapine and clozapine-*N*-oxide and to assess whether these metabolic pathways correlate with the production of reactive species.
Methods. Clozapine, *N*-desmethylclozapine and/or clozapine-*N*-oxide were incubated with hepatic- and cardio-selective CYP isoforms and cofactor (NADPH) in the presence of 2'-7'-dichlorofluorescein diacetate (DCF-DA) at 37°C for 60 minutes. Reactive intermediate ions were trapped using glutathione and cyanide and detected with liquid chromatography-mass spectrometry (LCMS). Clozapine, clozapine-*N*-oxide and *N*-desmethylclozapine were quantified using LCMS in the same incubations, and their rate of formation by each isoform was assessed relative to the formation of reactive species (measured indirectly via DCF-DA oxidation by free radicals).
Results. Isoforms favouring the formation of *N*-desmethylclozapine (CYP1A2, CYP2C19, CYP1A1) were not associated with the oxidation of DCF-DA, whereas isoforms favouring the production of clozapine-*N*-oxide (CYP3A4) demonstrated significant formation of reactive species. Interestingly, the reduction of clozapine-*N*-oxide to clozapine was catalysed by all isoforms studied. This process was associated with DCF-DA oxidation, particularly among cardio-selective enzymes that did not catalyse the oxidation of clozapine to clozapine-*N*-oxide (CYP1A1). Glutathione and cyanide trapping altered enzyme activity but the associated reactive intermediates did not correlate with overall reactive species.
Discussion. Unique to this investigation is the finding that various CYP isoforms catalyse clozapine-*N*-oxide reduction back into clozapine. This has allowed us to explore the relative contribution of oxidation and reduction pathways, alongside formation of reactive intermediates, to the production of non-selective reactive species. Identifying such species connects metabolism and oxidative stress as a potential mechanism behind clozapine-induced cardiotoxicity.

226. Preclinical behavioural recognition cages to detect health impacts of polypharmacy and deprescribing.

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Introduction. Polypharmacy (use of ≥ 5 drugs) and increasing DBI (measures exposure to anticholinergic and sedative drugs) is associated with impaired global health outcomes in observational studies. Due to residual confounding by indication, it is impossible to determine whether disease or medications are the cause. Novel automated preclinical behavioral recognition cages allows investigating drug and disease effects and measure daily functional activity.
Aims. Using automated behavioral recognition cages, we aim to determine the effect of monotherapy, polypharmacy, DBI and deprescribing (withdrawing drugs) on functional activity over 23 hours in healthy aged mice.
Methods. From 12 to 21 months of age, healthy male C57BL/6 mice were fed control or treatment containing therapeutic doses of five drugs with Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram), or single drugs from the High DBI polypharmacy regimen. At 21 months, animals either continued or had treatment deprescribed. At 24 months behavioral activity was measured using the LABORAS.
Results. Oxycodone, oxybutynin, and simvastatin monotherapy did not alter any of the 55 measures of function (11 activities in 5 different time segments within the 23hrs measured). Metoprolol (4/55 activities) and citalopram (13/55) decreased gait speed, and citalopram also reduced locomotion and climbing (p<0.05). For polypharmacy regimens, the number of outcome changes increased as DBI increased (Zero DBI; 1/55, Low DBI; 14/55, and High DBI; 23/55). For High DBI polypharmacy, from 11am-7pm (light period), an elevation in immobility, and reduction in locomotion, climbing, rearing and gait speed was observed but from 12am-7am (part of dark period), we observed increased locomotion, rearing, eating, drinking and gait speed (p<0.05). Deprescribing reversed most of these changes.
Discussion. Medications alone can cause functional impairment. The changes in daily functional activity vary between treatments but polypharmacy with high DBI induced the greatest functional impairment which was reversed with deprescribing. Preclinical automated behavioural recognition cages can be a useful tool to screen for global health impacts induced by clinically relevant drug regimens in ageing.

227. CD38 catalyses nicotinamide mononucleotide (NMN) base exchange

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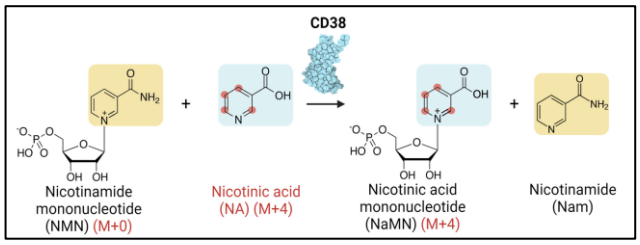

Introduction. Nicotinamide mononucleotide (NMN) is a biosynthetic precursor to nicotinamide adenine dinucleotide (NAD^+), a critical redox cofactor that declines with age. NMN is undergoing clinical development as a therapy for age-related disease, however PK/PD studies are complicated by background levels of this naturally occurring metabolite. One surprising aspect of NMN treatment is an acute increase in the metabolites nicotinic acid mononucleotide (NaMN) and nicotinic acid adenine dinucleotide (NaAD) – two metabolites from the *de novo* pathway, a completely different arm of NAD^+ biosynthesis. These metabolites act as highly sensitive biomarkers for exogenous NMN treatment that guide PK/PD studies, however it is unclear how NMN could increase levels of metabolites from an independent pathway.
Aims. To identify how exogenous NMN leads to an increase in the *de novo* pathway intermediates NaMN and NaAD, which we proposed was due to base exchange or deamidation activity of the enzyme CD38.
Methods. We used stable isotope labelling of NMN and nicotinic acid combined with targeted metabolomics to test whether CD38 mediated deamidation or a base exchange reaction on NMN.
Results. CD38 effectively catalyzes the exchange of the nicotinamide moiety of NMN a nicotinic acid, yielding NaMN. This base exchange reaction was confirmed both *in vitro* and *in vivo*, and treatment with a small molecule CD38 inhibitor *in vivo* completely abolishes the acute increase in NaMN and NaAD caused by NMN treatment.
Discussion. These findings suggest that CD38 connects the salvage and Preiss-Handler pathways for NAD production, which were previously thought to be independent pathways. This finding provides a mechanistic basis for the use of NaMN and NaAD as sensitive biomarkers for PK/PD studies of exogenous NMN treatment.

228. EP4, a novel mitochondrial uncoupler, reduces disease features in obesity-associated severe asthma

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Introduction. Obesity is a major risk factor for asthma, and obese asthma patients, particularly females, are more likely to have more severe and treatment-resistant disease. However, how obesity drives the pathogenesis and severity of asthma is poorly understood and there is an urgent requirement for new, more effective treatments that improve the quality of life for these individuals. Mitochondria-targeting uncoupling drugs have been shown to exert potent anti-obesity and insulin-sensitising effects in mice. We have developed a novel lipid-derived, mitochondria-targeting partial uncoupler called EP4 that overcomes safety issues caused by full uncouplers.
Aims. To assess the effects of oral administration of EP4 in clinically relevant mouse models of obesity and obesity-associated severe asthma.
Methods. Female wild-type BALB/c mice (*n*=8/group) were fed a high fat diet (HFD) or control chow for 9 weeks. Some groups of mice were then sensitised to ovalbumin (Ova; intraperitoneal) on day 0 (or saline control), followed by intranasal Ova challenges (days 12-13) to induce experimental asthma. Mice were then re-challenged with Ova (days 33-34) in the absence, or presence, of dexamethasone (DEX; 2mg/kg) to model inhaled corticosteroid therapy. Some groups of mice were administered EP4 (per oral) at low (1mg/kg) or high (10mg/kg) doses (days 21-34). Diets were continued during experimental asthma. Endpoints (day 35; week 14) included *in vivo* invasive plethysmography to measure airway hyperresponsiveness (AHR) and bronchoalveolar lavage to measure airway inflammation.
Results. HFD/obesity resulted in steroid-insensitive AHR in both the absence, or presence, of experimental asthma. Treatment with EP4 at 1mg/kg or 10mg/kg had minimal effects on Ova-induced airway inflammation. Interestingly, treatment with EP4 at 10mg/kg suppressed steroid-insensitive AHR in HFD/obesity-induced experimental asthma.
Discussion. Treatment with EP4 in HFD/obesity and HFD/obesity-induced experimental asthma reduces normally intractable AHR, justifying further investigation of EP4's therapeutic utility in obesity-associated severe asthma.

229. Pulmonary and vascular consequences of influenza A virus (IAV) infection in atherosclerotic mice

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Introduction. Influenza in people with atherosclerosis increases their risk of plaque destabilization, myocardial infarction, and death *via* largely unknown mechanisms.
Aim. To determine the effect of IAV infection on lung and vascular function and atherosclerosis progression in mice.
Methods. 5-wk old APOE$^{-/-}$ mice were placed on a high fat diet for 7 weeks. At the beginning of week 8, mice were intranasally inoculated with a low dose of the mouse adapted Hk-x31 strain of IAV to recapitulate a mild seasonal form of influenza disease. The degree of atherosclerosis was assessed at week 14 with oil-red O staining. Blood pressure and pulse rate were taken weekly. Myography was used to assess endothelial-dependent (Ach; 10^{-9}-10^{-5}M) and independent relaxation (sodium nitroprusside 10^{-5}M), respectively. Lung function and the mean linear intercept (MLI) to determine alveolar enlargement were assessed. The Fulton index was measured for right ventricular hypertrophy.
Results. Aorta from IAV infected mice failed to relax to both ACh and SNP and displayed an impaired contraction to the vasoconstrictor, U46619 indicating a substantial reduction in endothelial and smooth muscle function. Infected APOE$^{-/-}$ mice exhibited significantly reduced plaque coverage when compared to uninfected controls. Infected mice displayed significantly decreased FEV0.1/FVC ratios when compared to controls, and this was associated with right ventricular hypertrophy and significantly higher MLI. IAV also caused significant systolic hypotension and bradycardia during the acute phase of the infection.
Discussion. IAV infection in APOE$^{-/-}$ mice causes profound dysfunction of the aorta and pulmonary remodeling manifested as damage to the alveoli and lung dysfunction. IAV also caused bradycardia and hypotension and right ventricular hypertrophy, suggestive of pulmonary hypertension.

230. Investigating the pathophysiological effects of geography-specific landscape fires smoke

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Introduction. Millions of people are exposed to landscape fire smoke (LFS) globally and inhalation of LFS particulate matter is associated with poor respiratory and cardiovascular outcomes.
Aims. To characterize the pathophysiological effects of representative LFS airway exposure on respiratory and cardiac function and on asthma outcomes.
Methods. LFS was generated using a customized combustion chamber. 8-week old female Balb/C mice were administered physiologically relevant concentrations (low, 25µg/m^3 or moderate, 100µg/m^3, 24 hour equivalent) of LFS particulate matter (10µm and below; PM_{10}) daily for 3 (short-term) and 14 (long-term) days in the presence and absence of experimental asthma. Lung inflammation, gene expression, structural changes and lung function were assessed. 8-week old male C57Bl/6 mice were administered low concentration LFS PM_{10} for 3 days. Cardiac function and gene expression were assessed.
Results. Short- and long-term LFS PM_{10} airways exposure increased airways hyperresponsiveness and induced steroid-insensitivity in experimental asthma. Long-term LFS PM_{10} airways exposure also decreased gas diffusion. Short-term LFS PM_{10} airways exposure decreased cardiac function and the expression of gene changes relating to oxidative stress and cardiovascular pathologies.
Discussion. We have characterised significant detrimental effects of physiologically relevant concentrations and durations of LFS PM_{10} airways exposure on lung and heart function. Our study provides a platform for assessment of mechanisms that underpin LFS PM_{10} airways exposure on respiratory and cardiovascular disease outcomes.

231. Defining the cross-tissue communication between lungs and white adipose tissue in COPD

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Introduction. Cigarette smoke (CS) exposure is a major risk factor for chronic obstructive pulmonary disease (COPD) and its systemic comorbidities. White adipose tissue (WAT) plays a pivotal role in regulating inflammation and metabolism. However, the impacts of CS exposure and COPD on WAT remain poorly understood.
Aim. To explore how CS exposure impacts on WAT and its potential contribution to COPD.
Methods. Male BALB/c mice were exposed to either room air or CS for 8 weeks, with or without an antioxidant, apocynin (5 mg/kg/day). Indirect calorimetry and metabolic parameters were assessed throughout the experiment. At the end of the experiment, bronchoalveolar lavage fluid was collected to assess pulmonary inflammation, and WAT was excised to examine adipokine expression and function. Differentiated 3T3L1 adipocytes and lung epithelial cells were used to examine the effect of CS exposure *in vitro* and the possible link between the lung and WAT.
Results. CS exposure was associated with worsened glucose tolerance in the mice, despite weight loss and elevation of VO_2. In WAT, enhanced lipolysis, aberrant expression of key adipokines (*Apelin, Adiponectin, Resistin, TNFa, IL-6*) and increased protein carbonylation were detected. Inhibition of oxidative stress by apocynin prevented glucose intolerance, which was associated with preserved adipokine expression and WAT function, without significant effects on weight loss and VO_2 increase. 3T3L1 adipocytes were largely unresponsive to direct stimulation with CS extract. However, exposure to culture media derived from CS extract-treated lung epithelial cells elicited oxidative stress, aberrant adipokine expression and enhanced lipolysis similar to that of WAT *in vivo*.
Discussion. CS exposure may enhance resting metabolism, but this was paradoxically associated with WAT dysfunction and aberrant adipokine expression in an oxidative stress-dependent manner. WAT dysfunction and aberrant adipokine expression appears to be mediated by factors deriving from CS-exposed lung epithelial cells, supporting a lung-WAT axis of tissue crosstalk existence in COPD.

232. PF670462 attenuates stiffness and strain induced fibrogenesis in precision cut lung slices

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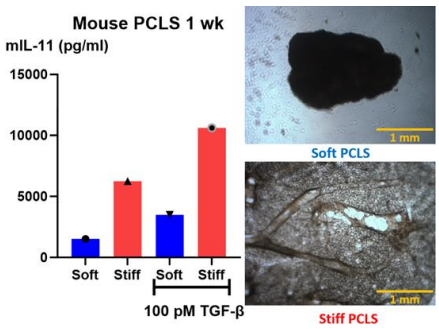
Introduction. Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease in which the alveoli become damaged and scarred, leading to a stiffening of the lung. Precision cut lung slices (PCLS) are powerful tools for the investigation of lung diseases. However, the current methods of inflating lungs with liquefied low-melting point agarose solution inevitably create a stiff/strained environment when the agarose solidifies, activating fibrogenesis pathways. PF670462 is a casein kinase 1δ/ε inhibitor that was previously shown to be a potent anti-fibrogenic agent will be used in this model to attenuate fibrosis.
Aims. To investigate the extent of fibrogenesis induced by agarose inflation and evaluate the therapeutic value conferred by PF670462.



Methods. Murine PCLS were inflated with gelatin and agarose to create a soft and stiff microenvironment respectively. Fibrogenesis markers were examined with both immunoassays and global proteomics. The therapeutic effects of PF670462 were benchmarked against nintedanib, pirfenidone and SB431542 using immunoassays and global proteomics.
Results. In a stiff/strained environment, the PCLS had heightened levels of fibrotic markers such as IL-11 both in the presence and absence of 100 pM TGF-β. PF670462 attenuated fibrotic markers like IL-11. PF670462 had complementary effect with nintedanib and superior efficacy as compared to pirfenidone and SB431542
Discussion. The stiff/strained microenvironment confers an innate fibrogenesis model that is sensitive to TGF-β. This is beneficial for studying IPF in PCLS, but it may confound studies that may not need fibrogenesis. PF670462 was shown to attenuate fibrogenesis in this model with complementary or superior effects compared to other antifibrotics.

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233. Investigating the application of an in vitro 3D cell culture technique to improve the translational potential of novel immunotherapy drug treatment strategies for pleural mesothelioma.

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Introduction. Pleural mesothelioma (PM) is a rare and aggressive cancer that develops in the mesothelial lining of the lung pleura following exposure to asbestos. Despite the recent availability of immunotherapy treatments, the median survival rate of PM patients is only 18 months, highlighting the urgent need for improved immunotherapy treatment strategies. There have been minimal advancements to current standard care due to inconsistencies between preclinical 2D model and clinical trial drug screening data. However, 3D cell culture systems that more accurately recapitulate the complex structure and microenvironment of mesothelioma tumours make them ideal models for pre-clinical screening of novel immunotherapy drugs.
Aims. To develop a novel 3D co-culture model of mesothelioma using HLA-matched patient-derived tumour cells and healthy volunteer-derived B&T cells to facilitate prospective preclinical immunotherapy drug screening.
Methods. Mesothelioma cell lines were HLA-matched with B&T cells isolated from healthy volunteer peripheral blood samples. Matched samples were then co-cultured on novel decellularized porcine lung scaffolds. Formalin-fixed scaffold sections were stained with clinically validated mesothelioma biomarkers (BAP1, CDK2NA, p53) and B&T cell markers (CD3, CD80) via histological assessment through immunohistochemistry (IHC) to confirm mesothelioma and immune cell co-existence.
Results. Four HLA-matches to two mesothelioma cell lines were obtained from 30 healthy volunteer blood samples. Cells were able to grow on and penetrate the porcine scaffold for up to 14 days. IHC staining of CD3 and CD80 cells confirmed the presence of B&T cells in our novel 3D model. Presence of cancer cells was also confirmed through the assessment of clinically validated mesothelioma biomarkers, including loss of BAP1 and CDK2NA expression, and the presence of p53.
Discussion. Our study successfully established a novel 3D scaffold cell culture model of mesothelioma that facilitates the co-existence of mesothelioma and immune cells. We anticipate this 3D mesothelioma model can be utilised for prospective preclinical immunotherapy drug screening studies.

234. “I feel like I’m working at Amazon”. Pharmacists’ experience of outpatient telepharmacy.

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Introduction. Telepharmacy has been used effectively as a complement to, or *in lieu* of, face-to-face care across the full range of the pharmacists’ role. The evidence on patient satisfaction for telepharmacy is largely positive. However, pharmacists' perceptions and experiences of telepharmacy remain under-explored.
Aims. To describe service activity at a metropolitan hospital outpatient telepharmacy before (August 2019-March 2020) and during (March-September 2020) COVID lockdown and explore pharmacists’ experiences and opinions.
Methods. A three-phase explanatory mixed methods design was used: (1) a retrospective audit to measure quantitative changes in telepharmacy activity, (2) interviews with pharmacists (n=10) to explain patterns uncovered in audit data and explore provider experiences of delivering the service, (3) process mapping to visualise work process changes.
Results. A total of 1442 outpatient telepharmacy occasions of service were delivered to 687 unique patients across the study period. Most patients were cardiopulmonary transplant recipients (68.2%). A 6-fold increase in occasions of service was observed during COVID-19 lockdown (208 vs 1234), with the median shipping distance decreasing from 266 km to 64 km ($p<0.001$) and express deliveries increasing 38-fold ($p<0.001$). Three themes were identified from interview data that illuminate pharmacists’ experience and perceptions of delivering the telepharmacy service: reduced efficiency and effectiveness compared to face-to-face care; shifting responsibility for medication management (from patient to pharmacist); and perceived value of the telepharmacy service (patient’s value convenience, pharmacists question sustainability of workload and cost).
Discussion. A metropolitan hospital outpatient telepharmacy service maintained pharmaceutical care during COVID-19 lockdowns. However, this came at the expense of reduced job quality and satisfaction for pharmacists. Consideration of patient suitability, economic implications, and required infrastructure is necessary to ensure that telepharmacy services are effective and sustainable for both patients and pharmacists.

235. Infliximab or adalimumab antidrug-antibody complex size has more clinical value than concentration

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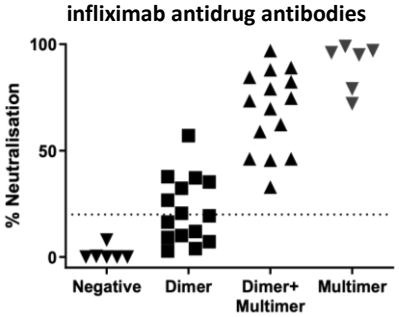

Introduction: The detection of anti-drug antibodies (ADA) to infliximab or adalimumab is an important element in the testing algorithm of therapeutic drug monitoring. There are a variety of methods to detect ADA and the titre of antibody is an output that is used in some algorithms. Use of the homogenous mobility shift assay (HMSA) to detect presence of ADA also provides a measure of the immune complex size formed when ADA bind to drug *in vitro*. Prior studies have demonstrated that large ADA complexes were neutralising. Distinguishing neutralising from non-neutralising ADA may have clinical benefit.
Aims: To determine whether immune complex size correlated with neutralising.
Methods: A review of the ADA test service results for infliximab and adalimumab using the HMSA method was completed. ADA positive samples were categorised according to the size of drug-ADA complex formed, ability to neutralise infliximab/adalimumab and presence of IgG4 ADA.
Results: The review of our laboratory infliximab and adalimumab test service results found that 52% of samples with drug concentration <2mg/L were ADA positive. Over 80% of ADA positive samples formed dimer complexes of drug and ADA *in vitro*, whereas 17% had a mix of dimer and multimer complexes and 3% had solely multimeric complexes. There was a strong correlation between drug neutralising effect and presence of multimer complexes, that also contained IgG4 isotype ADA (r^2=0.79). In contrast, drug neutralising effect was absent or weak in samples with only dimer complexes. The Figure shows the correlation between infliximab ADA complex size and neutralising capacity.
Discussion: Immune complex size correlated more strongly than ADA concentration for drug neutralising effect and, therefore, may be a better variable to use for dose adjustment or drug switching decisions in patients on infliximab or adalimumab.

236. Examining methods to assess busulfan exposure in paediatric stem cell transplant recipients.

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Introduction. Busulfan is a chemotherapy agent used in stem cell transplantation with a narrow therapeutic index.
Aim. To assess the ability of the dosing tool, NextDose®, to estimate busulfan exposure, and quantify the difference between estimation based on non-compartmental analysis (NCA) and model-based methods (MBM) by comparing (i) current clinical practice NCA methods and NextDose® NCA methods, (ii) current clinical practice NCA methods and NextDose® MBM, and (iii) NextDose® NCA and NextDose® MBM.
Methods. Pharmacokinetic data characterising intravenous busulfan usage in paediatric stem cell transplant recipients was obtained from hospital sites across Australia and New Zealand. Busulfan dose, concentration-time measurements, and patient-specific characteristics were entered into NextDose® to give NCA and MBM predictions of busulfan area-under-the-concentration-time-curve (AUC). Predictive performance was assessed through calculation of the relative bias (RE) and imprecision (RMSE) in the mean difference between each software protocol.
Results. 90 patients with a total of 2170 concentration-time measurements were included in the analysis. A mean difference in busulfan AUC estimates of 0.95 mg.h/L [95% CI: -5.83 to 7.72] was observed between clinical practice and NextDose® NCA; a mean difference of -8.30 mg.h/L [95% CI: -18.59 to 1.99] was observed between clinical practice and NextDose® MBM; and a mean difference of -9.24 [95% CI: 19.03 to 0.53] was observed between NextDose® NCA and NextDose® MBM. There was a statistically significant difference between both NCA methods and NextDose® MBM. The median RE [Interquartile Range] and RMSE between clinical practice and NextDose® NCA was -0.49% [-2.78 to 1.92] and 0.04 mg.h/L; between clinical practice and NextDose® MBM was 8.86% [6.88 to 8.86] and 0.13 mg.h/L respectively; and between NextDose® NCA and MBM was 10.58% [7.99 to 13.96] and 0.13 mg.h/L respectively.
Conclusions. MBM observed a significantly higher exposure estimate compared to NCA methods (difference of 8.30mg/mL.h between clinical practice and MBM, and 9.24mg/ml.h between NextDose® NCA and MBM) therefore the method of estimation used in exposure-response studies must be considered when determining exposure targets and implementing into clinical practice.

237. Pregnancy in patients receiving tacrolimus immunosuppression: relationship between blood and plasma tacrolimus concentrations and their association with gestational outcomes.

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Introduction: In patients receiving tacrolimus (Tac) immunosuppression during pregnancy (transplantation, lupus nephritis), trough concentrations in blood (BC_0) are monitored to minimise rejection and/or adverse effects. Since BC_0 decreases during pregnancy, dose is increased to maintain consistent BC_0. However, as Tac is highly bound to erythrocytes and plasma proteins, changes in BC_0 may reflect decreases in haematocrit during pregnancy, rather than changes in clearance. Measuring trough concentrations in plasma (PC_0) may minimise the effect of haematocrit.
Aims: To investigate the relationship between BC_0 and PC_0 and associations with pregnancy outcomes.
Methods: BC_0, biochemistry and outcomes were obtained from clinical records. PC_0 were measured by LC-MS/MS. Relationships between BC_0 and PC_0 were investigated by linear mixed effect models with log transformation, adjusting for repeated measures and covariates (dose, trimester, haematocrit, albumin, total protein). Effects of BC_0 and PC_0 on neonatal (birth weight, gestational age) or maternal (creatinine, dialysis, gestational hypertension, preeclampsia, severe hypertension, glomerulonephritis, diabetic nephropathy, reflux nephropathy) outcomes were investigated by linear regression or binary logistic regression. P-values were adjusted for multiple comparisons.
Results: In 9 participants median (range) BC_0 (n=97) and PC_0 (n=88) were 5400 (1600-23100) and 880 (275-4270) ng/L, respectively. There was an association between BC_0 and PC_0 ($P < 0.0001$). For every 100 ng/L increase in BC_0, mean PC_0 increased by 1.1% (mean ratio = 1.011, 95% CI: 1.008, 1.014). Haematocrit, albumin and total protein were not significant covariates. Higher plasma creatinine was associated with dose ($P<0.05$) and trimester ($P<001$). Infant birth weight was associated with BC_0 ($P<0.0004$). For every 100 ng/L increase in BC_0 median, mean infant birth weight decreased by 60 g (95% CI: -90, -30). There was no association of other maternal outcomes with BC_0 or PC_0.
Discussion: Routinely increasing dose to maintain constant BC_0 may be unwarranted, with high doses and BC_0 contributing to decreased maternal renal function and lower birth weights, respectively.

238. Can we predict saliva penetration of drugs?: a systematic review

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Introduction. Saliva is an emerging matrix for therapeutic drug monitoring (TDM).
Aims. To determine the physicochemical properties that influence the penetration of drugs from plasma to saliva.
Methods. Medline and Web of Science (1980−2023) were searched for human clinical studies, which determined drug pharmacokinetics in both saliva and blood. Studies with at least 10 subjects and 5 paired saliva-blood concentrations per subject were included. For each study, the ratio of the area under the concentration–time curve between saliva and total (protein-bound + unbound) blood was determined to assess penetration into saliva. Physicochemical properties of each drug (pKa, lipophilicity, molecular weight, physiological charge, hydrogen-bond donor–HBD, hydrogen-bond acceptor, polar surface area–PSA, rotatable bonds, fraction of drug unbound to plasma protein) were obtained from PubChem and Drugbank. Drugs were categorised by their ionisability and saliva-to-blood ratios were predicted with adjustment for protein binding and physiological pH via the Henderson-Hassenbach equation. Spearman correlation analyses were performed for each category to identify factors predicting saliva penetration (α=5%). Study quality was assessed by the Risk Of Bias In Non-randomised Studies–of Interventions (ROBINS-I) tool.
Results. Overall, 50 studies including 46 drugs (antipsychotics, antimicrobials, immunosuppressants, antithrombotic, anticancer, and cardiac drugs) were included. The median saliva-to-blood ratios were similar for drugs in the amphoteric, basic, and acidic groups (0.59, 0.50 and 0.43, respectively) and lowest for drugs in the neutral group (0.16). Higher penetration into the saliva of acidic group drugs was associated with lower ionisation and protein binding (predicted vs. observed ratios: r^2=0.85, P=0.009, n=6). For basic group (n=24), protein binding was the only predictor (P=0.02). For amphoteric group (n=10), HBD and PSA were predictors ($P<0.05$). For neutral group (n=6), no single factor could predict penetration into saliva (all $P>0.05$). All the studies had a low-to-moderate risk of bias.
Discussion. Many commonly used drugs penetrate saliva. For acidic, basic and amphoteric drugs, physicochemical properties can predict saliva penetration. Further research is required to evaluate the contribution of drug transporters and physiological factors influencing saliva penetration of drugs.

239. The clinical impact of urate self-monitoring to improve allopurinol persistence in gout.

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Introduction. Despite effective therapies for gout, non-persistence of urate-lowering therapy (ULT) is common. Whilst point-of-care urate measuring devices are available, self-monitoring by gout patients remains underexplored as a tool to improve persistence.
Aims. To assess the impact of a patient-led model-of-care utilising point-of-care urate monitoring on persistence to allopurinol, time within target urate concentration, and incidences of gout flares.
Methods. Participants were people with gout (N=31), currently taking allopurinol, living in Australia, who self-monitored their urate at least once monthly using a point-of-care device (HumaSens2.0^{plus}) for 12 months. Persistence was measured using medication event monitoring technology (MEMS®). Non-persistence was defined as ≥30 consecutive days without MEMS openings. Days within target urate (<0.36 mmol/L) were determined using linear interpolation. Relationship between using the device and both urate and time between MEMS openings was assessed using two-tailed non-parametric Spearman correlation ($p<0.05$). Gout flares were self-reported using Gaffo criteria.
Results. Most participants were male (94%) and below target urate at baseline (74%). Overall, seven participants presented repeated "missed doses" (≤2 allopurinol doses missed consecutively) and "drug holidays" (≥3 missed). While urate self-monitoring, 29 participants persisted with allopurinol treatment. Of the 2 who discontinued, one participant had recorded elevated urate (0.46 mmol/L) and two gout flares, while the other recorded low urate (0.31 mmol/L) in the absence of gout flares. Participant urate self-monitoring increased the time spent within target urate (79% to 100%, $p=0.3456$), and decreased the incidences of gout flares (8 to 5, $p=0.25$), with most occurring within the first 6-months. Additionally, for some (13%) participants, urate concentration decreased with device usage ($p<0.03$).
Discussion. A patient-led model-of-care using urate self-monitoring supported the maintenance of target urate concentrations and allopurinol persistence, and decreased incidences of gout flares. Further research on the feasibility of this model-of-care is warranted, alongside assessment in a larger randomised controlled trial.

240. Developing an optogenetic system to shine a light on intracellular GPCR signalling

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Introduction. G protein-coupled receptors (GPCRs) mediate vastly diverse responses. Accumulating evidence reveals they can also signal from intracellular membranes. Differential localisation of GPCRs at the cell surface or intracellular membranes may result in location-specific outcomes. A fundamental understanding of localised GPCR signalling is required, however several pharmacological methods (e.g., endocytic inhibitors) can have confounding consequences. We therefore turned to optogenetic methods.
Aims. Establish a targeted optogenetic rhodopsin β2-adrenoceptor chimera (opto-β2AR) (Siuda et al., 2015) in human embryonic kidney (HEK) 293 & human highly metastatic (HM) triple negative breast cancer, MDA-MB-231 HM, cells. Quantify light-mediated cAMP and ERK phosphorylation (pERK).
Methods. Opto-β_2AR was targeted to intracellular membranes using literature-sourced location sequences. Targeting was confirmed with confocal microscopy, line scan analysis and a custom ImageJ script. Light-mediated receptor signalling was quantified using cAMP and pERK assays.
Results. Opto-β_2AR was successfully targeted to early endosomes, Golgi and nucleus. In both cell lines, opto-β_2AR activation modulated pERK levels in opposite directions (fig. 1, n=4, error bars mean±SEM), analogous to its wild-type counterpart. There were also light-dependent cAMP increases in both cell lines with differential effects based on location.
Discussion. Light-activated targeted opto-β_2ARs can be used to understand location-specific GPCR signalling. Future studies will use this system to investigate a mechanistic basis as disease-relevant GPCR signalling may also be location-dependent. A greater understanding of this is likely to encourage new strategies for GPCR-targeted drug discovery.

Siuda ER et al (2015) Nat Commun 6:8480-8492

241. Inhibition of the AT4R/ IRAP as a novel strategy to reduce renal fibrosis and injury in chronic kidney disease

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Introduction. We have identified the angiotensin type 4 receptor (AT_4R), also known as the insulin regulated aminopeptidase (IRAP) as a promising new anti-fibrotic target, with pharmacological inhibition of IRAP reversing age-induced cardiac fibrosis. However, little is known about its role in the setting of renal injury and disease.
Aims. This study aimed to: 1) Investigate the effects of pharmacological inhibition or genetic deletion of IRAP in a high salt diet (HSD) induced model of kidney disease and 2) Compare the reno-protective effects observed with IRAP inhibition to current gold standard treatment with the angiotensin converting enzyme inhibitor (ACEi), Perindopril.
Methods. WT (C57Bl/6J) and IRAP KO mice (12 weeks old, n=6-10/group) were subjected to either normal drinking water (NDW) or 2% NaCl in drinking water (HSD) over a 12-week period. WT mice on a HSD were randomised to receive either: vehicle; IRAP inhibitor, HFI-419 (0.72mg/kg/day; s.c via osmotic mini-pump); or an ACEi, Perindopril (1mg/kg/day via drinking water) in the final 4 weeks of the experimental protocol.
Results. Mice fed a HSD presented with significantly increased IRAP expression (HSD+Veh=7.4±0.3% vs NDW=4.0±0.3%, $p<0.001$), interstitial fibrosis (HSD+Veh=4.1±0.3% vs NDW=2.7±0.3%, $p<0.001$) and glomerulosclerosis (HSD+Veh=2.3±0.2 vs NDW=1.7±0.2, $p<0.05$) which was accompanied by a trend in reduced kidney function. IRAP inhibition or gene deletion significantly reduced interstitial fibrosis (HSD+HFI-419=3.0±0.3%, HSD IRAP KO=3.0±0.2%; all $p<0.01$) and glomerulosclerosis (HSD+HFI-419=1.7±0.2, HSD IRAP KO=1.8±0.2, all $p<0.05$), accompanied by trends of improved renal function when it came to measurements of urinary urea as well as urinary and plasma creatinine levels. In contrast, Perindopril treatment had limited ability to improve either interstitial collagen expression (HSD+Perindopril=3.3±0.7%, $p>0.05$) or glomerulosclerosis (HSD+Perindopril=2.0±0.3, $p>0.05$).
Discussion. Targeting IRAP displayed an ability to regress fibrosis and glomerulosclerosis in a more long-term model of kidney disease, which was associated with trending improvements in renal function. Moreover, this study demonstrated that both pharmacological inhibition and genetic deletion of IRAP offered broader and greater reno-protection than treatment with the ACEi, perindopril.

242. Probing the molecular basis of P2X1 receptor activation and inactivation using cryo-EM

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Introduction. The P2X1 receptor is under-researched, with insufficient structural and pharmacological data and a notable absence of drug-like P2X1 receptor antagonists. Genetic validation in male mice showed 100% infertility, highlighting the P2X1 receptor's potential for male contraception.[1] The male contraception field desperately needs innovation, given the high rate of unintended pregnancies (over 300,000 daily) and the limited choice of only two male contraceptives.
Methods. Cryogenic electron microscopy (cryo-EM) was used to solve the human P2X1 receptor structure in an ATP-bound desensitised state and NF449-bound closed state. P2X1 receptor binders were validated using a HEK293 P2X1 expressing cell line in a calcium mobilisation assay and a radioligand binding assay. Additionally, calcium mobilisation assays were conducted on HEK293 cells expressing single residue mutants of the P2X1 receptor.
Results. Cryo-EM images of the P2X1 receptor revealed severe preferred orientation and optimisation efforts identified the secondary detergent, fluorinated FOS-Choline-8, which significantly reduced preferred orientation. These improvements resulted in a 1.96 Å and 2.61 Å structure of the P2X1 receptor in an ATP-bound and NF449-bound state respectively. P2X1 receptor antagonists, NF449, TNP-ATP are the most potent P2X1 receptor antagonists while MRS2159 and ATA are less potent but are better starting molecules for designing druglike P2X1 receptor antagonists for in vivo use. Single P2X1 receptor mutations produced significant increases in the EC50 of α,β-methylene ATP and decreases in the IC50 of NF449 compared to WT-P2X1.
Discussion. The next step is to generate novel, and improve current, P2X1 receptor antagonists leveraging our high-resolution P2X1 receptor structures.

1. White CW, Choong Y-T, Short JL, et al (2013) Male contraception via simultaneous knockout of α1A-adrenoceptors and P2X1-purinoceptors in mice. Proceedings of the National Academy of Sciences of the United States of America 110:20825–20830. https://doi.org/10.1073/pnas.1318624110

243. Neurogenic inflammation exacerbates urinary tract infection in mice.

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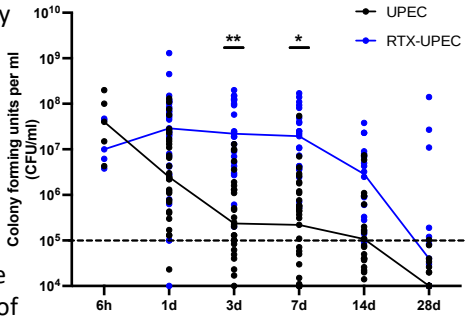

Introduction: Neurogenic inflammation (NGI) arises due to pro-inflammatory peptide release by sensory nerves. NGI of the bladder causes urinary symptoms, including urgency and pain, and disrupts the urothelial barrier. Urothelial barrier disruption is a key risk factor for developing chronic urinary tract infections (UTIs). However, whether NGI directly impacts UTI susceptibility has yet to be determined. **Aims**: We aimed to use mouse models to study the contribution of NGI to UTI severity and persistence. **Methods:** Resiniferatoxin (RTX) was instilled into the bladder lumen of female C57Bl/6J mice over 3 consecutive days to induce neuropeptide release from afferent nerves and NGI. NGI was quantified by histological evaluation of bladder sections and phenotyping immune cells via flow-cytometry. 1-day post-RTX treatment, uropathogenic *E. coli* (UPEC $1x10^9$CFU/ml) was instilled into the bladder lumen via bladder catheterization to induce UTI. UTI persistence and bacterial load was determined by measuring colony forming units (CFU) from urine, kidneys, and spleen over 28 days. **Results**: RTX significantly increased T cell, B cell, NK cell, neutrophil and macrophage infiltration into the bladder wall and disrupted bladder wall integrity (n=5, $p<0.005$), indicative of NGI. Installation of UPEC induced UTI that gradually resolved over 28 days in untreated mice (Figure). In contrast, RTX treated mice exhibited significantly greater bacterial load in urine over time (Figure) (n=5-40, $^{**}p<0.005$; $^{*}p<0.05$) and increased CFU in the bladder wall and kidneys at 3-and 7-days post-infection (n=5-10, $^{*}p<0.05$), indicating an exacerbated and prolonged UTI. **Discussion:** These results show that neurogenic inflammation can have a dramatic impact on the severity and persistence of UTI. These findings suggest that neuropeptides released from sensory nerves in the bladder may play a crucial role in the host-defence against UTIs. The mechanisms underlying these effects have yet to be determined but could be caused by interactions between neuropeptides and the immune cells that regulate infection clearance.

244. Exploring molecular determinants underpinning high potency of MIPS3215 at adenosine A_{2B} receptor

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Introduction. The adenosine A_{2B} receptor ($A_{2B}R$) is a pivotal therapeutic target for a range of diseases, including ischemia, myocardial infarction, cancer, and fibrosis (Vecchio et al, 2019). As endogenous adenosine has low affinity for the $A_{2B}R$, the majority of $A_{2B}R$ effects occur during stress or injury, which is typically associated with elevated local adenosine levels. There is a paucity of potent, selective, and high-efficacy $A_{2B}R$ agonists. We recently identified a selective $A_{2B}R$ agonist with the highest known potency, MIPS3215 (Awalt et al, 2022). MIPS3215 can provide key insights into the molecular determinants that underpin $A_{2B}R$ efficacy and selectivity and as such facilitate drug discovery efforts at this potential therapeutic target.
Aim. To elucidate the molecular basis of high $A_{2B}R$ potency of MIPS3215 using a combination of computational and pharmacological approaches.
Methods. Computational docking of MIPS3215 was performed using an active $A_{2B}R$ structure (PDB ID: 7XY7). Key mutant $A_{2B}Rs$ containing single alanine substitutions were stably expressed in FlpINCHO cells. ELISA, cAMP accumulation and intracellular calcium mobilisation assays were performed to quantify the changes in receptor expression and functional potency and efficacy of NECA, a non-selective $A_{2B}R$ agonist, and MIPS3251.
Results. Among 24 mutants tested, only four significantly reduced receptor expression level. Alanine mutations of non-conserved residues located in the top of transmembrane 7 (N273A) and extracellular loop 3 (K265A and K267A) critically impacted MIPS3215 potency and selectivity at the $A_{2B}R$, leaving NECA unaffected. Docking simulation predicted that the linker and allosteric moiety of MIPS3251 interact with these residues. Notably, K265A significantly changed the bias profile of MIPS3251, suggesting the role of the extracellular loop 3 in biased agonism at the $A_{2B}R$.
Discussion. This study has identified critical amino acid residues for the high potency and selectivity of MIPS3215 at the $A_{2B}R$, contributing to a profound understanding of an efficient probe for the investigation of the $A_{2B}R$.

Awalt JK et al (2022) J Med Chem 65:9076-9095.
Vecchio EA et al (2019) Pharmacol Ther 198:20-33.

245. Meropenem and ciprofloxacin combination regimens against isogenic *Pseudomonas aeruginosa* strains with different resistance mechanisms in a dynamic hollow fibre model

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Introduction. *Pseudomonas aeruginosa* has a large armamentarium of mutational resistance mechanisms enabling resistance emergence during therapy against almost all antibiotics in monotherapy, including novel β-lactam/β-lactamase inhibitors.
Aims. To evaluate dosing regimens of meropenem (MER) and ciprofloxacin (CIP), alone and combined, against isogenic *P. aeruginosa* strains with different resistance mechanisms in a dynamic hollow fibre infection model (HFIM).
Methods. Four isogenic *P. aeruginosa* strains were: PAOD1 (spontaneous *oprD* mutation/loss of porin OprD), PAΔADmexR (*ampD* knock-out/AmpC overexpression and *mexR* knockout/MexAB-OprM overexpression), PAOD1ΔmexR and PAOD1ΔAD (other arrangement of combinations of the resistance mechanisms). Dosing regimens were: MER continuous infusion (CI, 6g daily dose against all strains, 12g daily dose additionally against MER-resistant strains), CIP intermittent infusions (400mg, 8-hourly [Q8] as 1-h infusions), and both combinations.
Results. All monotherapies resulted in regrowth with amplification of MER- and CIP-resistant subpopulations. The combination regimens suppressed total and resistant counts of PAOD1, PAΔADmexR and PAOD1ΔAD to below the limit of counting. Against PAOD1ΔmexR, the MER 6g CI + CIP regimen performed synergistically from 24h to 120h, while MER 12g CI + CIP was synergistic from 24h to 192h. MER-resistant counts displaying small colony morphology emerged from 72h and 168h with the respective combination regimens, plateauing at values similar to the control; CIP-resistant small colonies emerged from 216h with the low-dose combination only.
Discussion. Combination regimens of MER and CIP enhanced bacterial killing and suppressed regrowth and resistance of strains with one (PAOD1) or two (PAΔADmexR, PAOD1ΔAD) resistance mechanisms. Even against the double-resistant PAOD1ΔmexR substantial synergy occurred up to 120 or 192h. The performance of the combination regimens depended on the different resistance mechanisms present.

246. Targeting adenosine receptors for the treatment of myocardial infarction and heart failure

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Introduction. Myocardial infarction (MI) is the leading cause of morbidity and mortality worldwide. To date, many G protein-coupled receptors (GPCRs) have been used as therapeutic targets for many diseases, including cardiovascular diseases. The GPCR family known as the adenosine receptors represent promising therapeutic targets to minimise cardiac damage and pathological remodelling post-MI. However, from preclinical models, the cardioprotective efficacy of prototypical agonists can decrease in high-risk populations, particularly advanced age (Gao et al, 2000). Adenosine A_1 receptor (A_1R) biased agonists, such as VCP746, can preferentially stimulate cardioprotection in the absence of the dose-limiting on-target unwanted effects of hypotension and bradycardia (Valant et al, 2014).
Aims. To further explore the therapeutic potential of A_1R biased agonism, this study assessed the influence of the cardiovascular risk factor, advanced age, on *in vivo* cardioprotection and haemodynamic effects mediated by prototypical and biased adenosine receptor agonists in a rat model of acute ischaemia-reperfusion injury (IRI).
Methods. Ischaemia (30-min) by left anterior descending coronary artery ligation followed by reperfusion (120-min) were performed in aged rats (70-72 weeks old). A single bolus dose of NECA (prototypical/non-biased adenosine receptor agonist) or VCP746 were infused upon reperfusion. Haemodynamics, including heart rate and blood pressure, were measured throughout the procedure using a Millar pressure catheter.
Results. At reperfusion, VCP746, but not NECA, significantly reduced infarct size in aged rats. NECA significantly reduced blood pressure and heart rate upon infusion, whereas VCP746 had no effects on haemodynamics.
Discussion. A_1 receptor biased agonist, VCP746, successfully reduced myocardial infarct size following from acute IRI without causing unwanted on-target side effects in aged rats. Our study demonstrated the limitation of adenosine receptor prototypical agonists and highlighted the benefits of adenosine receptor biased agonists in specific disease settings, such as MI associated with advanced age.
[1]Gao F *et al* (2000) Am J Physiol Heart Circ Physiol 279(1), H329-338
[2]Valant C *et al* (2014) PNAS 111(12), 4614-4619

247. Improving the therapeutic application of an anti-fibrotic peptide by conjugation to nanoparticles.

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Introduction. Cardiac fibrosis is a key driver of left ventricular (LV) remodelling and dysfunction in heart failure but is ineffectively treated by currently-available therapies. Recombinant human relaxin (Serelaxin; RLX) has emerged as a rapidly acting anti-fibrotic peptide therapy but requires continuous infusion via osmotic minipump (Pump-RLX) delivery or daily injection to maintain its activity, owing to its short *in vivo* half-life of ~4-8h. As RLX is also poorly gut absorbed, identifying drug delivery vehicles that can overcome these limitations could help enhance its clinical application.
Aim. To conjugate RLX to biodegradable glycine-coated super-paramagnetic iron oxide nanoparticles (SPION-RLX) and evaluate its activity and oral applicability in a murine model of isoprenaline (ISO)-induced cardiomyopathy.
Methods. 11-12-week-old male C57BL/6 mice (n=8/group) were subjected to daily s.c-injections of ISO (25mg/kg) or saline (vehicle) for 5 consecutive days before being left untreated until day 14. Sub-groups of ISO-injured mice were either left untreated or administered with Pump-RLX (via 7-day minipumps), SPION-RLX administered i.p (every 72 hours) or via daily drinking water (p.o), or with Empty-SPIONs alone, from days 7-14 post-injury. Measures of LV inflammation, hypertrophy, remodelling, fibrosis, and function were then carried out at day 14 post-injury.
Results. ISO-injured mice presented with LV inflammation, cardiomyocyte hypertrophy, interstitial fibrosis, vascular rarefaction, and systolic dysfunction at day 14 post-injury, compared to their saline-injected counterparts. Glycine coated-SPIONs were taken up by infiltrating immune cells and directed to the site of injury (LV) for release. This allowed SPION-RLX to be detected in the circulation of treated mice at similar levels to Pump-RLX treatment. Strikingly, i.p or p.o-administered SPION-RLX significantly attenuated several measures of LV inflammation, remodelling, fibrosis, and dysfunction to an equivalent extent as Pump-RLX, after 7-days of treatment. However, these therapeutic effects of SPION-RLX were not induced by Empty-SPIONs alone.
Discussion. These findings revealed that the conjugation of RLX to glycine coated SPIONs improved its longer-term activity and oral applicability, which paves the way for its clinical application and those of other peptide therapies.

248. Dissecting endothelin-dependent signalling to identify new targets for fibrosis-related cardiac dysfunction

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Introduction. Endothelin is a key regulator of cardiovascular function, with profound vasoconstrictor and direct inotropic effects on the heart. Despite promising preclinical data, clinical trials using endothelin receptor antagonists for heart failure have been disappointing, likely due to the dual functions of endothelin in the heart and vasculature. Our data implicate endothelin signalling at the centre of cardiac fibrosis and inflammation that lead to contractile dysfunction (Voges et al, 2023), through signalling mechanisms that remain unclear.
Aims. To use systems biology approaches to define the endothelin signalling network in human cardiac organoids.
Methods. Pluripotent stem cell-derived human cardiac organoids (hCO) represent a controlled functional model that is ideal for investigating intracellular and paracrine signalling. hCO were stimulated with endothelin-1 (100 nM), fibrotic mediators and cardiac inotropes for analysis of cardiac function and downstream signalling responses using global proteomics and phosphoproteomics. Contractile force, rate and kinetics were assessed using Matlab scripts. Statistical analyses and visualisation of (phospho)proteomics data were performed using Proteome Discoverer (v2.3) and R.
Results. Endothelin-1 induced potent concentration-dependent increases in force and rate in hCO (EC50 ~9 nM), as did other cardiac inotropes (noradrenaline and histamine). However, unlike noradrenaline and histamine, endothelin prolonged relaxation time, which is indicative of diastolic dysfunction. Endothelin-1 stimulation also promoted fibrosis-related changes in extracellular matrix protein expression (e.g. fibronectin, tenascin C) to the same extent as angiotensin II. Comparative analyses of endothelin-1 and histamine global phosphoproteomics data identify known and novel signalling mediators (e.g. PALLD and PDE4D) that may account for opposing effects on contraction kinetics.
Discussion. Our functional and initial phosphoproteomics data suggest divergent signalling responses for endothelin-1 and other cardiac ligands. We are pursuing the mechanisms that underpin these differences using systems biology approaches including time-resolved global phosphoproteomics and network modelling. These studies will uncover downstream endothelin signalling nodes that could represent new targets for cardiac dysfunction in heart failure.

Voges H et al (2023) Cell Rep. 42(5):112322.

249. Exploring A_{2B} receptor regulated human cardiac fibroblast phosphoproteome for fibrosis target discovery

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Introduction. There remains unmet therapeutic need to treat pathological cardiac fibrosis to reduce heart failure progression. The A_{2B} receptor, a G protein-coupled receptor, can modulate second messengers and decrease the expression of transforming growth factor beta 1 (TGF-β1), a pro-fibrotic mediator, in cardiac fibroblasts (Vecchio et al, 2016). Mapping the complex downstream phosphorylation events following A_{2B} receptor activation in human cardiac fibroblasts will refine our understanding of the potential of the A_{2B} receptor as a therapeutic target to treat fibrosis.
Aim. To delineate the global phosphorylation networks downstream of A_{2B} receptor activation in adult human ventricular cardiac fibroblasts (CFs) in the presence or absence of the pro-fibrotic mediator, TGF-β1.
Methods. Quantitative data-dependent acquisition mass spectrometry (DDA-MS) phosphoproteomics was employed to map the global protein phosphorylation events in CFs (Lonza, Switzerland) following agonist exposure, NECA (7 min; 0.28 μM) ± TGF-β1 (48 h; 10 ng/mL). Phosphorylated peptides were quantified and identified using MaxQuant software package. A web based Phospho-Analyst platform was used for statistical and gene ontology analysis. Benjamini-Hochjberg test (adjusted p value of 0.05) was used to determine significantly regulated phosphoproteins.
Results. 260 phosphosites mapping to 238 proteins were downregulated with NECA treatment compared to vehicle control, whereas 155 phosphosites (on 138 proteins) were upregulated. Following TGF-β1 treatment, NECA induced significant downregulation of 67 phosphosites mapped to 62 proteins whereas 35 phosphosites (on 35 different proteins) were upregulated compared to vehicle control. Gene ontology analysis showed overrepresentation of NECA regulated phosphoproteins in biological processes including actin-myosin structure organization and regulation of cytoskeleton in the absence and prescence of TGF-β1, respectively.
Discussion. A_{2B} receptor activation in human CFs regulates phosphoproteins involved in cytoskeleton and actin-myosin structure organisation. Future analysis will evaluate the subsequent influence on the modulation of fibrosis.

Vecchio EA *et al* (2016) Biochem Pharmacol 117:46-56.

250. Single target-dual therapy at M4 muscarinic receptors for the treatment of schizophrenia

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Introduction. Activation of the M4 muscarinic acetylcholine receptor (mAChR) has emerged as a promising approach for the treatment of schizophrenia, with anticipated outcomes beyond those provided by any currently approved antipsychotic medication. This is particularly highlighted by the fast progress of two distinct drug candidates for the M4 mAChR into advanced stages of clinical trials, xanomeline[1], an orthosteric agonist, and emraclidine[2], a positive allosteric modulator. Whilst targeting the same receptor, xanomeline and emraclidine do so via distinct regions and mechanisms. Xanomeline and emraclidine may soon become the first antipsychotic medications with novel mode of action for the treatment of schizophrenia in almost 50 years. But can a "single target-dual therapy" approach, where both xanomeline and emraclidine are co-administered, provide further therapeutic benefits?
Aims. We aimed to measure the synergistic effect between xanomeline and emraclidine at the M4 mAChR across multiple signalling pathways and validate this effect in a preclinical model predictive of antipsychotic efficacy.
Methods. We used a wide range of cell-based assays in recombinant systems expressing the human or mouse M4 mAChRs for quantification of synergistic effect, and the NMDA antagonist MK-801-induced locomotor activity test in C57Bl6J mice for assessment of reversal of psychotic symptoms.
Results. Across all signalling pathways investigated, emraclidine can significantly "boost" xanomeline agonist properties at both human and mouse M4 mAChRs. *In vivo*, low-dose of xanomeline (1 mg/kg) has no significant effect on hyperlocomotion, whilst low-dose of emraclidine (30 mg/kg) only partially reverses hyperlocomotion. Excitingly, combining these two clinical candidates together, xanomeline (1 mg/kg) and emraclidine (30 mg/kg), can fully reverse MK-801-induced hyperlocomotion, with less side effects than when administered independently at high-dose.
Conclusion. Whilst xanomeline and emraclidine may be the next generation *first-in-class* antipsychotics, we now have evidence that co-administration of an M4 mAChR agonist and an M4 mAChR positive allosteric modulator has the potential to become the next *best-in-class* antipsychotic medication for the treatment of schizophrenia.

[1] Clinical trial ID: NCT04659161; [2] Clinical trial ID: NCT05227690

251. Exploring cell senescence in iAstrocytes and iMicroglia from ALS patients

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Introduction. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition with no cure, resulting in upper and lower motor neuron death, and an average lifespan of 3-5 years following diagnosis. Cell senescence is a non-proliferative state that cells enter due to prolonged stress, while remaining metabolically active. Under chronic conditions, this can lead to increased inflammatory and oxidative stress in healthy neighbouring cells. Senescent cells also dysregulate signalling pathways allowing them to evade apoptosis, making them a difficult target for drug design. Post-mortem motor and prefrontal cortices from ALS patients display upregulated senescence-associated proteins, such as p16, p21, and p53, which are additionally correlated with region-specific motor neuron death. Similar trends are seen when measuring senescence using senescence-associated beta galactosidase (SA-β-Gal) staining in cells derived from ALS patients, and in animal models of ALS. Although this evidence implicates senescence in ALS, whether this occurs secondary to motor neuron degeneration or is cell autonomous is unclear.
Aims. This study aims to characterise senescence in ALS astrocytes in the absence of motor neuron degeneration, and to explore underlying molecular pathways.
Methods. The project utilised three experimental assays to characterise cell viability, proliferation, and senescence expression in iPSC-derived astrocytes developed from fibroblast lines donated by ALS patients carrying *c9orf72* hexanucleotide repeats (n=3) and age-matched healthy controls (n=3). To explore some molecular pathways underlying iAstrocyte senescence in ALS, we performed western blotting for the Bcl-xL protein, which helps senescent cells evade apoptosis.
Results. ALS iAstrocytes showed 2-fold and 4-fold significantly reduced cell viability and proliferation ($p<0.05$), respectively, while displaying 2-fold significantly more senescent cells compared to controls ($p<0.05$). ALS iAstrocytes showed 2-fold significantly higher Bcl-xL expression compared to controls ($p<0.05$).
Discussion. These data confirmed the presence of senescence in human ALS astrocytes and identified a potential target for further molecular pathway exploration and drug design.

252. Indications for pharmacogenomic testing in Australia

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Introduction. Pharmacogenomic (PGx)-guided prescribing can improve the efficacy and safety of some medicines. Numerous international guidelines for PGx testing exist but there are no Australian PGx guidelines.
Aim. To determine the indications for PGx testing in Australia.
Methods. A national multidisciplinary working group was established under governance of the Royal College of Pathologists of Australasia (RCPA). Evidence on PGx-guided prescribing from the Clinical Pharmacogenomics Implementation Consortium (CPIC), the Dutch Pharmacogenomics Working Group (DPWG), the US Food and Drug Administration (FDA), Australian Medicines Handbook (AMH), prescribing information (PI) for individual medicines, published literature and other sources was considered by the working group. Indications for PGx testing were classified into three categories based on working group consensus. *Recommended*: adverse effects or therapeutic failure can cause significant patient harm and international guidelines consistently recommend testing. *Consider:* risk of adverse effects or therapeutic failure is significant and international guidelines generally recommend testing. *Available*: association with adverse effects or therapeutic failure, but no consensus from international guidelines regarding testing.
Results. Pharmacogenomic testing is *recommended* for abacavir, allopurinol, azathioprine, carbamazepine, capecitabine, clopidogrel, fluorouracil, mercaptopurine, oxcarbazepine, phenytoin, and voriconazole. *Consider* testing for amitriptyline, atomoxetine, citalopram, codeine, nortriptyline, tamoxifen, tramadol and warfarin. Pharmacogenomic testing is *available* for atorvastatin, clomipramine, doxepin, escitalopram, fluvastatin, imipramine, lansoprazole, omeprazole, pantoprazole, paroxetine, pravastatin, rosuvastatin, sertraline, simvastatin and tacrolimus. No other medicines currently have sufficient evidence for PGx-guided prescribing to support PGx testing.
Discussion. A pragmatic consensus-based approach was used to determine the indications for PGx testing in Australia based on international guidelines and multidisciplinary national PGx expertise. The indications will be made available on the RCPA website. Review of the indications for PGx testing in Australia will occur annually.

253. Factors associated with antipsychotic use in people with dementia in Australia.

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Introduction. Antipsychotics are commonly used to manage behavioural and psychological symptoms of dementia; however, their use may vary due to patient sociodemographic factors.
Aims. To identify patient factors associated with antipsychotic use in people with dementia.
Methods. This was a cross-sectional study using linked 2021 Australian Census and Pharmaceutical Benefits Scheme (PBS) data. Medications were coded according to the Anatomical Therapeutic Chemical (ATC) Classification system, with psychotropics and antipsychotics defined as ATC N and N05A, respectively. Dementia status was defined based on self-reported dementia diagnosis in the 2021 census and/or any dispensing of one antidementia medication from 2016 to 2021. Prevalence of antipsychotic use in 2021 was reported descriptively. Age, gender, education, number of comorbidities, need for assistance for core activities, language spoken, and living arrangements were included in a multivariable logistic regression model to explore factors associated with the use of antipsychotics in people with dementia.
Results. A total of 177,809 people with dementia were included (median age 84 [IQR: 78-89], 59% female. Most of the included people with dementia required assistance with core activities (79%), and had at least one other comorbidity (68%). In 2021, more than half of the people with dementia used at least one psychotropic medication (68%) and more than one in three were using two or more psychotropic drug classes (35%). Overall, 32,666 (18%) of people with dementia were using antipsychotics. Those with higher education (Bachelor degree or higher) (aOR: 0.74, p<0.001) and not requiring assistance for core activities (aOR: 0.51, p<0.001) were less likely to be prescribed an antipsychotic. Conversely, living in a non-private dwelling (such as a nursing home) (aOR: 2.83, p<0.001), and speaking a language other than English (aOR: 1.27, p<0.001) increased the likelihood of being prescribed an antipsychotic.
Discussion. Almost 1 in 5 people with dementia were using an antipsychotic. A range of sociodemographic factors were associated with antipsychotic use, with greater use in those with lower education, from culturally and linguistically diverse backgrounds, and requiring greater assistance. Further research is needed to explore the underlying reasons for differences in antipsychotic use in these at-risk populations.
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McCartney P (2001) J J 56:23-33
Starr R et al (2005) Pharmacology of FAB-4, ed Ono Y. pp 12-23, Tokyo, Abbey Road Press

254. Trajectories of oral bisphosphonate use after hip fractures: a population-based cohort study

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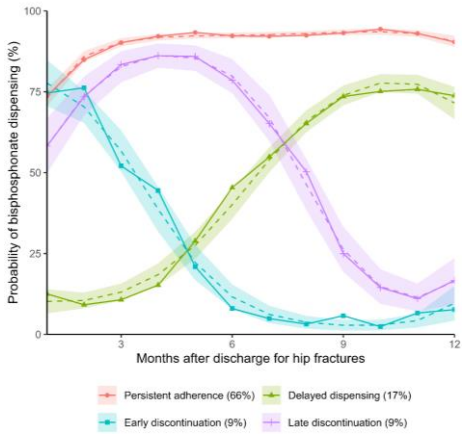
Background. Suboptimal antiresorptive use is not well understood but may be associated with poor outcomes following a first hip fracture.
Aims. To investigate the trajectories of oral bisphosphonate use following first hip fractures and the factors associated with different trajectories.
Methods. We conducted a population-based cohort study of all patients aged ≥50 years dispensed two or more bisphosphonate prescriptions following their first hip fractures in Victoria, Australia from 2012 to 2017. Twelve-month trajectories of post-discharge bisphosphonate use were identified using group-based trajectory modelling. Factors associated with having trajectories other than persistent adherence trajectory were assessed using multivariate multinomial logistic regression.
Results. We identified four trajectories of oral bisphosphonate use in 1,811 patients using oral bisphosphonates following first hip fractures:



persistent adherence (66%); delayed dispensing (17%); early discontinuation (9%), and late discontinuation (9%). Pre-admission bisphosphonate use was associated with a lower risk of delayed dispensing in both sexes (relative risk [RR] 0.28, 95% confidence interval [CI] 0.21-0.39). Older patients (≥85 years old versus 50-64 years old, RR 0.38, 95% CI 0.22-0.64) had a lower risk of delayed dispensing. Males with anxiety (RR 9.80, 95% CI 2.24-42.9) and females with previous falls had increased risk of early discontinuation (RR 1.80, 95% CI 1.16-2.78).
Discussion. Two-thirds of patients demonstrated persistent adherence to oral bisphosphonates over 12 months following hip fracture. Efforts to further increase post-discharge antiresorptive use should be sex-specific and address possible persistent uncertainty around delaying treatment initiation.

255. Association of the Drug Burden Index exposure with outcomes: a systematic review

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Introduction. The Drug Burden Index measures the cumulative exposure to anticholinergic and sedative medication.
Aims. To investigate the association of the DBI with clinical and prescribing outcomes in observational pharmaco-epidemiological studies, and the effect of DBI exposure on functional outcomes in pre-clinical models.
Methods. A systematic review was performed. A literature search of nine electronic databases, citation indexes and grey literature was performed (1 April 2007 to 31 December 2022). Studies that reported primary data on the association of the DBI with clinical or prescribing outcomes conducted in any setting in humans aged ≥18 years or animals were included. Quality assessment was performed using the Joanna Briggs Institute critical appraisal tools and the Systematic Review Centre for Laboratory animal Experimentation risk of bias tool.
Results. Of 2382 studies screened, 70 met the inclusion criteria (65 in humans, five in animals - mice). In humans, outcomes reported included function (n=56), cognition (n=20), falls (n=14), frailty (n=7), mortality (n=9), quality of life (n=8), hospitalisation (n=7), length of stay (n=5), readmission (n=1), other clinical outcomes (n=15) and prescribing outcomes (n=2). A higher DBI was significantly associated with increased falls (11/14, 71%), poorer function (31/56, 55%) and cognition (11/20, 55%) related outcomes. Narrative synthesis was used due to heterogeneity in study population, setting, study type, definition of DBI and outcome measures. In mice, outcomes reported included function (n=18), frailty (n=2) and mortality (n=1). In pre-clinical studies, a higher DBI caused poorer function and frailty.
Discussion. A higher DBI may be associated with an increased risk of falls, worse function and cognition. No firm conclusions can be made regarding other outcomes. Human observational findings are supported by preclinical interventional studies. The DBI may be used as a screening tool to identify older adults at higher risk of harm. Homogeneity in the method of reporting of the DBI in future studies may improve the ability to synthesise results.

256. Symptomatic and preventive medication use in people with Alzheimer's disease: 10-year study

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Introduction. Priorities of care among people with Alzheimer's disease (AD) may transition over time from intensive treatment of chronic diseases to focus on symptomatic care and preserving function for better quality of life.
Aims. To investigate longitudinal changes in symptomatic and preventive medication use among community-dwelling people with and without Alzheimer's disease (AD) five years pre- and post-AD diagnosis.
Methods. Retrospective cohort study involving 58,496 people with AD and 58,496 matched comparators without AD in Finland. Medication dispensing data of people with physician-verified AD diagnoses were obtained from the Finnish Prescription Register. Prevalence of symptomatic and preventive medication use were evaluated every six months from five years before to five years after AD diagnosis.
Results. During the one year before diagnosis, people with AD had the largest increase in people taking ≥3 symptomatic medications (+4.4% vs +2.2%) and ≥3 preventive medications (+6.4% vs +2.9%) compared to people without AD. Over the five years after diagnosis, the proportion of people taking ≥3 symptomatic medications stabilised around 37.1-39.8% in both cohorts; meanwhile, the proportion of people taking ≥3 preventive medications decreased by 6.0% in people with AD

but increased by 6.1% in people without AD. Over the 10 years, people with AD had a large absolute percentage increase in prevalence of antipsychotics (+22.7% vs +1.8%) and antidepressants (+19.1% vs +5.0%) compared to people without AD. During the same period, paracetamol and calcium supplement use increased by 31.1% and 20.4%, respectively. Among people with AD, the largest absolute percentage decrease in prevalence of preventive medications five years after diagnosis were beta-blockers (-9.8%) and statins (-7.0%).
Discussion. AD diagnosis is the key timepoint for change in symptomatic and preventive medication use. Medication assessments at the time of and following AD diagnosis appear to coincide with discontinuation of preventive medications but minimal changes in the prevalence of symptomatic medications.

257. Real-world evidence of antipsychotic utilization in Australia (2000–2021) using two datasets

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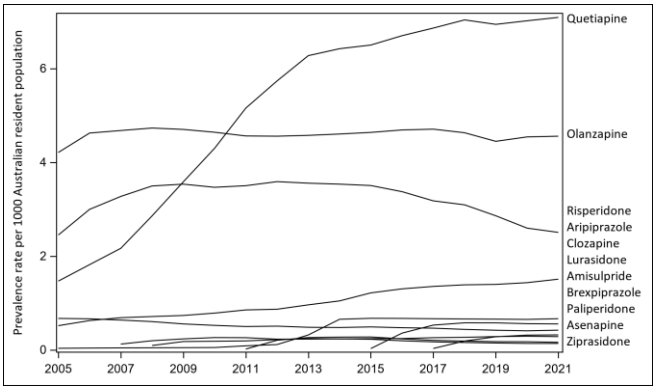

Introduction. Antipsychotic utilization is increasing globally, with significant off-label prescribing.
Aims. To determine antipsychotic utilization patterns in Australian adults, with a focus on on-label and off-label prescriptions.
Methods. We summarized trends in antipsychotic usage from PBS (Pharmaceutical Benefits Scheme) 10% dataset containing patient-level information on medicines dispensed in Australia between 2005–2021. We analysed diagnostic data for antipsychotics from BEACH (Bettering the Evaluation And Care of Health), a cross-sectional national survey from 2000–2016 consisting of data from general practitioner-patient encounters.
Results. We observed steady increases in both incidence and prevalence of antipsychotics, with an annual growth rate of 6.6%, mainly attributed to second-generation antipsychotics. As shown in the figure, quetiapine, olanzapine and risperidone were the most commonly prescribed. Among the patients receiving quetiapine, 35% were given the 25mg low dose without titration, with a median treatment duration of 85 (IQR 84–193) days. Analysis of diagnostic indications from BEACH indicated that 27% of antipsychotic prescriptions were off-label for indications such as depression, dementia, anxiety and insomnia, at much lower prescribed daily dosages.
Discussion. Each dataset adds a unique perspective to the concerning trend of increased antipsychotic utilization in Australia, with a significant proportion of off-label use. This could have a cascading effect on the development of adverse effects; more studies are required to understand the risks.

258. Time course of biomarkers of inflammation following birth and surgery

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Introduction: C-reactive protein (CRP) and procalcitonin are widely studied biomarkers for the diagnosis of infection. The plasma concentration of these biomarkers also increases, without infection, following birth and surgery. Understanding the time course of these biomarkers following these events in non-infected patients may provide a time and patient specific reference ranges for non-infected patients.
Aims: To describe the time course of CRP following birth and surgery.
Methods: CRP measurements from non-infected neonates, infants and adults following birth (Fukuzumi et al., 2016), and surgery (Davidson et al., 2013; Linnarsson et al., 2023; MacFater et al., 2022). CRP was described using a one compartment turnover model with first order elimination and volume of distribution of 2.5L/70kg. Birth and surgical event stimuli were described with one compartment, a zero-order input and first order elimination. CRP production, RateIN, was assumed to increase linearly with stimuli associated with birth, initial surgery, open chest period and delayed surgical chest closure. Covariates included postmenstrual age (empirical sigmoid emax), theory based allometric size using total body weight, factors related to severity of disease or surgical indication and route of birth (c-section vs vaginal). Models were developed using NONMEM 7.5.1.
Results: 1750 CRP concentrations were measured in 434 patients. Population parameter estimates were CRP CL 1.96 (95% CI: 1.86-2.09) L/day/70kg, CRP RateIN 0.772 (95% CI: 0.68-0.83) mg/day/70kg, birth effect stimulus elimination half life (Tel) 1.3h (95% CI: 1.18-1.45), surgical effect Tel 38h (95% CI: 36.6-41.1). The birth effect slope for stimulation of CRP was 19900

(95% CI: 17947-21952), larger than for the initial surgical effect (745; 95% CI: 668-773). Birth effect slope in neonates born via c-section was 5.1% of those born via vaginal delivery (95% CI: 5.06-7.98).
Discussion: Birth increases peak CRP concentrations as high as a 3 hour duration surgical event. However, surgical stimulus half-life was 29 time longer than for a birth stimulus. This study quantifies the time course of CRP following non-infectious events and may help identify patients with increased CRP concentrations associated with infection.

Davidson J et al (2013) Pediatr Res 74(4): 413-419
Fukuzumi N et al (2016) Sci Rep 6(1): 23871
Linnarsson C et al (2023) Paediatr Anaesth 33(7): 571-576
Macfater W S et al (2021) Ann Surg 275(1): e30-e36

259. Association between frailty and polypharmacy or multimorbidity in hospitalised adults

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Introduction. Multimorbidity is common in middle-aged and older adults. This often leads to polypharmacy due to the prescription of multiple guideline-directed medicines. Frailty commonly co-occurs with multimorbidity and is associated with an increased risk of medication-related harm.
Aims. To determine the prevalence of polypharmacy and multimorbidity in hospitalised middle-aged and older adults with and without frailty.
Methods. We conducted a cross-sectional study of middle-aged (45-64 years) and older adults (≥65 years) with inpatient admissions at the Royal Adelaide Hospital and Queen Elizabeth Hospital between 1 January 2022 and 30 June 2023. Long term medicines being taken at the time of discharge were extracted from the Sunrise Electronic Medical Record (Allscripts). Comorbidities were assessed using hospital coding data. Frailty was identified using the hospital frailty risk score (Gilbert et al., 2018). The association between frailty and polypharmacy or multimorbidity was assessed using the chi-squared test. Analyses were performed using R Statistical Software (version 4.1.2).
Results. 24,821 patients were identified over the study period. 79.4% (n=19,708) were exposed to polypharmacy, 72.6% (n=18,019) had multimorbidity and 47% (n=11,672) had frailty. Those with frailty were more likely to have polypharmacy (n=9,985, 85.5%) than those without frailty (n=9,723, 73.9%) (P<0.05). Those with frailty were more likely to have multimorbidity (n=10,926, 93.6%) than those without frailty (n=11,563, 87.9%) (P<0.05).
Discussion. Most hospitalised patients were exposed to polypharmacy and had multimorbidity. Approximately half of all patients had frailty. Frailty was associated with both polypharmacy and multimorbidity. Pharmacoepidemiologic studies are needed to quantify the risks and benefits of guideline-line directed medicines in individuals with multimorbidity and frailty.
Gilbert T et al. (2018) Lancet 391(10132): 1775-1782.

260. A prospective audit of incidence and management of aspiration events in patients with acute poisoning

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Introduction. Decreased level of consciousness is common in patients presenting to hospital with acute overdose. It is a risk factor for aspiration of gastric contents, and complications of aspiration include pneumonitis or pneumonia. Data describing adherence to existing guidelines are limited, yet it is important given the frequency of acute poisonings and antimicrobial stewardship principles.
Aims. To quantitate the incidence of aspiration events in acute poisonings and conduct a drug use evaluation in antibiotic prescribing in such patients at a tertiary hospital.
Methods. A prospective review of every presentation with acute poisoning to the Emergency Department between 1st March 2023 and 31st July 2023. Cases were identified via a purpose-built database operated by the clinical toxicology service. Electronic medical records were searched for other data. We recorded clinical features of aspiration, and the diagnoses and management by treating clinicians. Aspiration events were categorised, and indications for antibiotic prescribing, were based on the Antibiotic Therapeutic Guidelines (eTG). Outcomes from aspiration events at the time of discharge or death were determined.
Results. There were 316 presentations, 78 (25%) of whom had a decreased level of consciousness (GCS < 9), 13(4%) were intubated, 107 (34%) were admitted to hospital, including 46 (15%) to the intensive care unit. 5(0.02 %) had clinical

features of an aspiration event, ten of whom were diagnosed by the treating doctor as having aspiration pneumonia, and 10 were prescribed antibiotics. 2(0.006%) of other patients were prescribed antibiotics for indications including upper respiratory and urinary tract infection. On reflection of eTG, only three cases fulfilled diagnostic criteria for aspiration pneumonia. Ten patients were prescribed antibiotics (11 intravenous, of these 3 had intravenous plus oral), and in four of these the specific antibiotic was consistent with eTG (amoxycillin or benzylpenicillin), others prescribed were ceftriaxone and piperacillin/tazobactam. Of the ten patients prescribed antibiotics, in only one case both the indication and antibiotic selection was consistent with eTG. This related to inappropriate antibiotic duration and antibiotic choice. Discussion. Aspiration events are relatively common with acute poisoning but few progress to aspiration pneumonia so antibiotic prescribing is rarely required. Management of the cases was mostly appropriate and in accordance with current guidelines.

261. High-fructose diet starting from juvenile age provokes brain' tissue biochemical disturbances and changes sexual behavior phenotype in male rats

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Introduction. Excessive fructose-containing beverage consumption is common among youth. Our previous results indicate that a high-fructose diet (HFD) causes reproductive impairment in male rats. A relationship between metabolic changes in the brain and male sexual dysfunction could be supposed. We aimed to assess HFD effects from youth to puberty on brain tissue biochemistry and sexual behavior in rats.
Methods. Juvenile male albino rats were divided into 2 groups: control and HFD (replacement of drinking water with 10% fructose starting from postnatal day 23 up to 83). Adenyl nucleotide contents and pro/antioxidant system components in the brain, as well as hormones and sexual behavior, were investigated.
Results. It was shown that HFD altered brain metabolic pathways involved in mitochondrial bioenergetics: decrease in the content of ATP, ADP, and AMP were registered. Such disturbances were accompanied by the clear tendency for an increase in the level of brain tissue lipid peroxidation and depletion of the reduced glutathione pool. Additionally serum content of ceruloplasmin (the main extracellular antioxidant) significantly decreased. Male rats on HFD experienced a significant 28% drop in serum testosterone, with a twofold rise in LH and FSH levels. Alteration of sexual behavior can be mediated by both metabolic changes in neurons and hormonal regulation: HFD rats showed fewer mounts, ejaculations, and genital grooming compared to controls. As for preparatory mating indicators, they generally mirrored the rats' sexual activity effectiveness, but without statistical significance. Analysis of behavior patterns indicated altered locomotor and emotional reactivity in HFD group rats. Fructose-fed males displayed reduced vertical activity (34%), increased grooming (54%), and higher defecation (59%) in the open field test. Lastly, the male fertility index in the HFD group was lower than in the control.
Discussion. Collectively, these findings show that HFD can cause alterations at the biochemical level of the brain tissue, which are accompanied by changes in sex hormones and sexual behavior. Future research in this field will be indispensable for obtaining valid approaches to overcome the problem.

262. Acute toxicity of Thai PM 2.5 in zebrafish embryos

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Introduction. PM2.5 has been shown to induce more severe health effects than larger particulates. The average dust concentration in Thailand exceeded the standard that could be hazardous to human health. However, the effects of PM2.5 collected in Thailand on living organisms have not been studied. Zebrafish (*Danio rerio*) has been used as a model for studying human disease pathogenesis from environmental risks.
Aims. To investigate the acute toxicity and examine the underlying mechanisms of standard PM2.5 and PM2.5 collected at Chakri Naruebodindra Medical Institute in zebrafish embryos (Thai PM2.5).
Methods. Acute toxicity study of PM2.5 in zebrafish embryos is followed OECD test guideline no. 236. Zebrafish embryos were exposed to the chemicals at doses of 200-1,000 µg/mL for 96 hpf. Gene expression involved in oxidative stress, inflammation, and apoptosis were investigated.

Results. Standard PM2.5 and Thai PM2.5 dust induced mortality, malformations, and gene expression (oxidative stress, inflammation, and apoptosis) changed of zebrafish embryos. The LC_{50} values of standard PM2.5 and Thai PM2.5 were more than 1,483.27 µg/mL and 512.01 µg/mL, respectively. Thus, Thai PM2.5 caused more toxicity than standard PM2.5.
Discussion. The difference in toxicity from both samples in embryos is likely caused by the difference of dust components rather than polycyclic aromatic hydrocarbon and heavy metals. PM2.5 toxicity studies in zebrafish embryos can provide valuable information that may be useful to understanding the health hazards associated with PM2.5 exposure in humans, particularly in terms of its effects on early development and potential long-term health consequences. This information can be applied to human health risk assessment and environmental policy decisions.

263. Predicting the arrhythmogenic risk of drugs from the hypothetical 'worst case' drug

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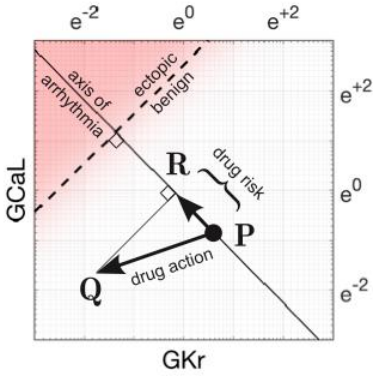
Introduction. All drugs in preclinical development must be assessed for arrhythmogenic risk in animal hearts or *in vitro* cell preparations. The standard tests primarily measure the potency of block of the cardiac hERG channel. More recently, it has been proposed that computer simulations of cardiac electrophysiology, which incorporate multiple ion channels, are more accurate predictors of risk. However, these models are difficult to apply outside of specialist computing laboratories.
Aims. We propose a new method to predict arrhythmogenic risk of a drug without running drug-specific simulations. The risk is instead predicted by comparing the drug's multi-channel block to that of a hypothetical 'worst case' arrhythmogenic drug which we identified using computer simulation.
Methods. Thousands of simulated cardiomyocytes were analysed to identify the shortest pathway to arrhythmia (ectopic beats) in the space of ion channel conductance parameters (Figure). That pathway, which we call the axis of arrhythmia, represents the action of the hypothetical drug. Once identified, the axis serves as a yardstick for quantifying the risk of new drugs without the need to run new simulations. We tested the predictions on a dataset of 109 drugs with known potencies of ion-channel block and clinical risk labels.



Results. Our method predicted the clinical risk with 88.1% to 90.8% accuracy, depending on concentration.
Discussion. The accuracy of our method compares favourably to existing computer methods (Passini et al, 2017) but without the technological barriers to adoption. It offers a simple pen-and-paper safety assay that is derived from computer simulation but does not involve simulation in its application.

Passini E et al (2017). Human In Silico Drug Trials Demonstrate Higher Accuracy than Animal Models in Predicting Clinical Pro-Arrhythmic Cardiotoxicity. Front Physiol 8: 668

264. Identification of Novel Toxicophores that Target Mitochondrial Respiratory Chain Complexes to Inform Drug Safety

Marion MacFarlane, MRC Toxicology Unit, University of Cambridge, Cambridge, UK

Disruption of mitochondrial function is a common cause of adverse drug reactions (ADR) with mitochondrial toxicity thought to be responsible for up to 50% of post-market drug withdrawals. Mitochondria possess a wealth of structural moieties and functional features, which can be targeted by a compound and lead to toxicity; these include Electron Transport Chain (ETC) inhibition, uncoupling of oxidative phosphorylation and alterations in mitochondrial dynamics. This poses a major health and economic burden, highlighting the need for better predictive models of mitochondrial toxicity. We have explored the potential for off-target mitochondrial liabilities in anti-cancer therapeutics and anti-psychotics and identified novel 'toxicophores' that target mitochondrial respiratory complex I. First, we showed that the anti-cancer drug mubritinib does not inhibit HER2 as reported, but directly inhibits mitochondrial respiratory complex I. Quantitative structure–activity relationship (QSAR) analysis using a library of chemical variants of mubritinib showed that modifying the 1H1,2,3-triazole altered complex I inhibition, thus identifying the heterocyclic 1,3-nitrogen motif as the toxicophore. We identified the same toxicophore in a second anti-cancer therapeutic carboxyamidotriazole (CAI) and showed that CAI also functions through complex I inhibition (Stephenson et al, eLIFE 2020). More recently, by combining clinically-relevant drug concentrations with multi-scale model systems, we discovered that the 'third-generation' anti-psychotic drug aripiprazole induces mitochondrial toxicity. Aripiprazole, brexpiprazole and cariprazine directly inhibit respiratory complex I through its ubiquinone-binding channel, further highlighting that thirdgeneration antipsychotics (TGA) acting as

partial dopamine receptor agonists exhibit off-target mitochondrial liabilities (Hardy et al, Biol Direct 2023). Building on our recent work showing that certain drug 'toxicophores' result in off-target inhibition of the ETC, we now aim to provide in silico models to enable better prediction of mitotoxicity. Machine learning (ML) provides a means for cost-effective, versatile and efficient prediction of toxic endpoints. However, the development of ML predictive models of ETC complex inhibition has yet to be established. I will highlight our current approach to building of predictive ML models for mitochondrial toxicity and ETC complex inhibition, in addition to the elucidation of common toxicophores, which may be flagged for safety concerns during drug discovery.

300. Assessment redevelopment to improve task authenticity and evaluation of critical analysis skills

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Introduction. Prior to 2017, the major assignment component for the 3rd year Pharmacology Unit, Drugs in Health & Disease (PHA3021, ~100 students) was a written essay (1000 words; 10% of unit mark) based on a chosen research topic. Students had difficulty engaging with the activity and appreciating its relevance. The submitted essays offered minimal critical evaluation of the topic.
Aims. To create a novel and authentic task that would effectively and rigorously enable students to demonstrate their ability to source and critically evaluate literature-based information relevant to current topics in pharmacology and to communicate their ideas to an appropriate scientific audience.
Methods. The iterative process began in 2017 with students tasked with creating a 1000-word Commentary article and accompanying graphical abstract, based on a recently published pharmacology paper. Students were provided with an Introductory Video and a sample annotated commentary. Over the next three years, scaffolding for the task was cumulatively provided through additional learning activities; firstly, with the introduction of a workshop on the Critical Analysis of a Scientific Paper, then a mid-assignment review consisting of formative in-class activities, and finally a summative mid-assignment report followed by a dedicated review Zoom meeting with the topic mentor.
Results. Student feedback at the end of the 2017 semester indicated recognition of the relevance of the assignment and the skills it developed; student skills in the critical analysis component of the task, however, required further development (mean mark=70.5±1.4%). The Critical Analysis workshop in 2018 led to some improvement in academic performance (mean mark=71.5±1.3%) but student feedback showed they were still wanting further support. The formative mid-assignment review in 2019 resulted in a small increase in performance (mean mark=72.4±1.4%), reflective of improved critical analysis skills, while the summative mid-assignment report and review meetings in 2020 culminated in a marked improvement in academic performance (mean mark=77.3±1.0%).
Discussion. These innovative changes in the nature of the assessment task and associated learning activities, lead to a marked improvement in student engagement and academic performance over a multi-year period that has remained consistent to date. Furthermore, this case-study demonstrates the iterative nature of assessment design; a process that requires yearly reflection and revision of practice in response to feedback from students and peers.

301. Antidepressants for pain in older adults: preliminary results from a systematic review

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Introduction: Pain is a common symptom experienced especially by older people and adds to their burden of diseases. Although not specifically intended for pain, antidepressants are a mainstay for treating many pain conditions.
Aims: To review the efficacy and safety of the different classes of antidepressants, compared to other medicines or placebo, for non-cancer pain in older adults (aged ≥65 years, including mean/median age ≥65 years).
Methods: Randomised controlled trials of older adults, published from inception to 3 August 2023, were retrieved through a comprehensive search of 12 databases: MEDLINE, Embase, CINAHL, PsycINFO, Cochrane Library, International Clinical Trials Registry Platform, Global Health, African Journals Online, Latin American and Caribbean Health Sciences Literature, Index Medicus for the Eastern Mediterranean Region, South East Asia Journal of Medical Sciences, and the China National Knowledge Infrastructure. (PROSPERO ID: CRD42023408204)

Results: 12 trials (N=932, age range 65-87 years) were included in our review. Duloxetine (5/12 trials) and amitriptyline (5/12 trials) were the most examined antidepressants. Postherpetic neuralgia was the most common pain condition among the trials (4/12). Efficacy in providing pain relief for all antidepressants in the included trials was inconsistent as 4/12 trials reported no significant reduction in pain compared to placebo/comparator. Serious adverse events leading to increased discontinuation/withdrawal of participants from trials or statistically significant adverse outcomes in the antidepressant group vs. comparator group were reported by 7/12 trials.
Discussion: Our review highlights the underrepresentation of older adults in trials which can limit the evidence to inform clinical practice. Large and more inclusive trials are needed to avoid fragmented care in this vulnerable population.

302. The development of non-hallucinogenic antidepressants inspired by psychedelic natural products

William Jorgensen, Psylo Bio, Sydney

Psylo has used a combination of high-throughput virtual screening and rational drug design to develop a library of non-hallucinogenic 5-HT2A agonists with antidepressant-like activity in rodent models. This presentation will describe our unique approach to the identification of new chemical matter with 5-HT2A activity, as well as lead optimization efforts leading to our clinical lead series. Details of the medicinal chemistry, 5-HT (and off-target) receptor profiling, in vitro neuroplasticity, in vivo pharmacokinetics, and behavioural pharmacology of this novel therapeutic class will be described.

303. Low dose psychedelics: Promise or placebo?

Vince Polito1 , Paul Liknaitzky2 . School of Psychological Sciences, Macquarie University1 , Sydney, NSW, Australia; Department of Psychiatry & Turner Institute for Brain and Mental Health, Monash University2 , Melbourne, VIC, Australia.

Introduction. The use of low doses of psychedelic substances (microdosing) is attracting increasing interest. Proponents of microdosing claim a wide range of clinically relevant cognitive enhancement effects. However, ongoing questions around effective doses and the role of expectation have led to confusion and controversy in microdosing science. Aims. To help unpack these issues I will present results of two recent reviews summarising all empirical microdosing research until April 2023, including a set of infrequently cited studies that took place prior to prohibition. Methods. Specifically, we reviewed 52 studies published since 1955, and summarised reported effects across five categories: neurobiological, physiological, phenomenological, affective and cognitive effects. Results. Studies showed a wide range in risk of bias, depending on design, age, and other study characteristics. Laboratory studies found changes in pain perception, time perception, conscious state, mood, and neurophysiology. Self-report studies found changes in cognitive processing and mental health. Discussion. I will discuss methodological issues in microdosing research, and in particular will evaluate recent claims that microdosing may be a placebo effect. I aim to highlight differences between acute and enduring microdosing effects, and provide some specific design suggestions to facilitate more rigorous future research.

304. Psychedelic assisted psychotherapies for alcohol and other drugs

A/Prof Jonathan Brett, St Vincent's Hospital, Sydney

With substantial government support and public interest in supporting research into psychedelic assisted psychotherapy, Australia is well positioned to start conducting such trials. This presentation will provide a research update on trials of psilocybin assisted psychotherapy for the treatment of substance use disorders and describe experiences conducting a world first Australian trial to study psilocybin psychotherapy for methamphetamine use disorder.

305. Jungle medicines: ayahuasca inspired psychedelic drugs for clinical mental health and addiction treatment

A/Prof Daniel Perkins, Psychae Therapeutics and The University of Melbourne

Ayahuasca is a sacramental beverage with a long history of use in Amazonian spiritual and healing rituals, and has been gaining increased scientific attention as a potential source of psychiatric therapeutics. This complex brew, traditionally made from a combination of two Amazonian plants, contains powerful psychoactive compounds, notably N,N-Dimethyltryptamine (DMT) and several harmala alkaloids. Like other classic psychedelics DMT is known to induce alterations in mood and perception primarily via the 5–HT2A receptor pathway, however, several additional mechanisms of action have been associated with both DMT and the brews harmala alkaloids, positioning it as a potential 'multi-target'

drug. This presentation will delve into the potential clinical applications of ayahuasca-inspired medicines for mental health and substance use disorders, the theorized mechanisms of action, and inherent complexities associated with developing a standardized pharmaceutical medicine from such a multi-plant, multi-constituent substance.

306. Advancing therapeutic development for cardiovascular disease with a preclinical human model of heart tissue

Dr Shiang (Max) Lim, St Vincent's Institute of Medical Research

Heart disease remains the leading global cause of mortality, necessitating the development of targeted and effective drug candidates for human patients. Unfortunately, progress in this field has been hindered by the absence of appropriate pre-clinical human models that capture the cellular diversity present in human heart tissue, as well as the inadequate translation of findings from animal studies to human contexts. In vitro human cardiac tissue models published thus far have been deemed too simplistic, lacking crucial non-cardiomyocyte cell populations essential for understanding cardiac health, disease mechanisms, and responses to pharmacological agents. To address these limitations, we have successfully engineered an advanced multicellular cardiac organoid model that integrates cardiomyocytes and non-cardiomyocytes derived from human induced pluripotent stem cells. This innovative pre-clinical human heart tissue model exhibits enhanced complexity, closely mirroring the cellular heterogeneity observed in native human heart tissue. As a result, our organoid model enables accurate cardiac disease modeling, facilitating drug/toxicology testing, and expediting drug development and discovery. By utilizing this cutting-edge organoid model, we anticipate a more efficient and targeted approach to drug development for heart disease, paving the way for improved clinical translation of pharmacotherapy and ultimately advancing human health and wellness.

307. Amnion cell-based therapies in cardiovascular disease

Prof Christopher Sobey, La Trobe University

Stroke is a major global health issue, especially in the aged, and yet has limited treatment options for long-term recovery. We have found that intravenous (iv) infusion of human amnion epithelial cells (hAECs) exerts beneficial effects when given within 2 h after stroke onset in mice, by limiting brain injury and inflammation, and reducing functional impairment [1]. Furthermore, a Phase I trial in 8 patients with acute stroke has established the safety and maximum tolerable dose [2]. We have continued our preclinical program to identify the time window post-stroke in which hAECs continue to provide long-term functional (motor or cognitive) benefit. Male and female C57Bl6 mice (12- 16 mo old) are given photothrombotic stroke in the left primary motor cortex or prefrontal cortex under inhaled isoflurane anaesthesia. At 1 or 7 d after stroke, mice received vehicle (saline) or $1x10^6$ hAECs iv. Motor function was assessed prior to surgery and at 1 and 8 w post-stroke using the cylinder task. Cognitive impairment was assessed using the Barnes maze test at 4 w post-stroke. hAECs were isolated from placentas donated by healthy volunteers who underwent elective Caesarean section delivery. Mice treated with hAECs at 1 d after stroke exhibited ~40% milder motor impairment at 1 w than vehicle-treated controls and this effect of hAECS therapy was sustained for 8 w. Interestingly, female (but not male) mice treated with hAECs at 7 d post-stroke exhibited much milder (~80%) motor impairment than vehicle-treated controls after 8 w. Furthermore, when hAECs were injected at 1 d following prefrontal stroke, those mice exhibited no significant cognitive impairment at 4 w compared with vehicle-treated controls. By contrast, extracellular vesicles from hAECs must be injected within 2 h of stroke to exert protective effects. In parallel studies we have also found that hAECs can inhibit angiotensin II-dependent increases in blood pressure, aortic stiffness and cognitive impairment. Our data suggest that systemic administration of hAECs and their extracellular vesicles can exhibit therapeutic effects in chronic cardiovascular disease and stroke.

References 1. Evans MA, Lim R, Kim H-A, Chu HX, Gardiner-Mann CV, Taylor KWE, et al. Acute or delayed systemic administration of human amnion epithelial cells improves outcomes in experimental stroke. Stroke 2018;49:700-709 (2018). 2. Phan TG, Lim R, Krause M, Chan ST, McDonald H, Gan P-Y et al. Phase I trial of amnion cell therapy for ischaemic stroke (I-ACT). Frontiers in Neuroscience 2023 May 9;17:1153231. doi: 10.3389/fnins.2023.1153231.

308. New viral technologies for manipulating cardiac cell function

Dr Melissa Reichelt, The University of Queensland

Neuregulin (NRG) administration in patients suffering from heart failure improves contractile function, however, systemic administration is dose limited by liver toxicity and has the potential to promote tumour growth. Adeno associated viruses (AAVs) represent a powerful tool for manipulating gene expression in a targeted, cell-specific manner and may offer a way to further refine NRG therapy. We developed an AAV construct utilising an AAV9 capsid combined with a chicken troponin t (cTNT) promoter to drive secretion of neuregulin from cardiomyocytes (AAV-NRG). Administration of this virus in P1 neonates significantly increased cardiac mass without evidence of pathological hypertrophy (i.e., increased collagen, changes in cardiac stress markers) within 9 days. We next explored the compatibility of recent improvements in gene targeting of cardiomyocytes including an enhancer for the cTNT promoter, an anti-miR and a new MyoAAV capsid. Together these refinements permit targeting of AAVs exclusively to cardiomyocytes. Cardiac fibroblasts also represent an important target for therapy in heart failure and ischemic heart disease. We have developed a high-throughput AAV screen capable of assaying 82 AAV virus coat (capsid) variants and we have identified two lead candidates that efficiently transduced cardiac fibroblasts; AAVHRS1 and HRP5. We have completed a preliminary validation of AAVHRS1 to confirm cardiac fibroblast transduction. Finally, we have optimised a method for transient AAV-driven expression of a self-deleting cre recombinase as a superior method for initiating gene deletion floxed mice. A construct was designed wherein a sequence for cre recombinase was flanked by loxp sites ensuring transient expression of Cre while conferring gene deletion when co-expressed with a floxed gene of interest. Together, AAVs represent a powerful toolbox for precise targeting of cardiac cells to unravel the mechanisms that regulate cardiac function and reduce side effects associated with systemic drug administration.

309. The role of extracellular vesicles in cardiometabolic disease

Nimna Perera, Monash Institute of Pharmaceutical Sciences

Diabetic cardiomyopathy is characterised by changes to left ventricular function and structure evident in diabetic patients independent of other cardiovascular disease. In early stages of diabetic cardiomyopathy, manifestation of structural changes includes left ventricular hypertrophy, cardiomyocyte apoptosis and left ventricular fibrosis (Ritchie and Abel, 2020). These pathophysiological changes and remodelling of the heart contribute to the diastolic dysfunction that is conducive to heart failure. The field of extracellular vesicles (EVs) is currently considered a trending topic in the biological sciences, where many research groups are exploring their therapeutic potential in disease. Unlike many drug treatments, EVs have potential advantages as therapeutics due to their stability, owing to lipid bilayers around vesicles which tolerate handling, immunogenicity, ability to cross blood barriers and exosomal efficacy after systemic delivery. In attempts to harness these benefits, an important study is the administration of exercise-derived vesicles into a db/db mouse model of type 2 diabetes to examine whether exercise-derived vesicles confer cardioprotection in type 2 diabetes. Exercise has shown to have benefits in glucose uptake, myocardial metabolism and improvements to insulin sensitivity, which can attenuate aberrations seen in diabetic cardiomyopathy. We are also still yet to identify what is contained within extracellular vesicles. Our lab is currently working on exploring EVs isolated from plasma in rest and exercise settings in humans and mice. Exercise is conceivably the most potent physiological activator of the fuel-sensing enzyme, AMPactivated protein kinase (AMPK), in many tissues in mammals. We have performed mass-spectrometry-based proteomic analysis on human EV samples following exercise indicating presence of AMPK isoforms $\alpha 1$, $\beta 1$, $\beta 2$, $\gamma 1$ and $\gamma 2$. We have also shown that through an AMPK-activated kinase assay, exercise-derived EV-rich fractions following sonication, induced a significantly large increase in kinase activity compared with sedentary EV-rich fractions. Importantly, activity in the non-sonicated EV-rich fractions from both mice and humans, showed reduced kinase activity. This work mainly examines how exercise-derived plasma EVs may provide potential benefit to cardiovascular diseaserelated diabetes mellitus and can lead to exciting future experimentation, where possible mechanisms can be explored on exactly how exercise-derived EVs confer cardioprotection in a diabetic heart. Harnessing the therapeutic effects of exercise-derived EVs may offer new insights to manage diabetes-related cardiovascular disease.

310. Drug-drug interactions arising from modulation of drug metabolising enzyme activities: Mechanisms and in vitro – in vivo extrapolation

John O. Miners, Discipline of Clinical Pharmacology, Flinders University College of Medicine and Public Health, Adelaide, SA, Australia.

Drug-drug interactions (DDIs) arising from decreased (inhibition) or increased (induction) enzyme (e.g., cytochrome P450 (CYP), UDP-glucuronosyltransferase) activities result in higher and lower exposure of the victim drug, respectively. In turn, this increases the probability of drug-related adverse effects (inhibition) and decreased efficacy (induction). Indeed, inhibitory DDIs have resulted in the withdrawal of several drugs from the market. Thus, DDIs represent both a clinical problem and a potential economic loss for the pharmaceutical industry. As will be described in this presentation, there have been significant advances in the prediction of the DDI liability of new chemical entities (NCEs) from in vitro data (in vitro – in vivo extrapolation; IV-IVE) over the last two decades. Further, IV-IVE has proven valuable for elucidating the mechanisms of DDIs involving established drugs. With respect to enzyme inhibition, the inhibition constant (Ki) and the fractional contribution (fm) of the affected enzyme to the overall metabolic clearance of the victim drug are the key experimental variables required for prediction of the increase in AUC using the equations for the basic and static mechanistic models of reversible inhibition. These models can accommodate inhibition of both hepatic and extrahepatic enzymes, which is an important consideration for DDIs involving CYP3A4. Investigation of time-dependent inhibition (e.g., mechanism-based inhibition) of CYP enzymes is additionally undertaken as part of the assessment of DDI liability. Standardised experimental conditions for characterisation of the induction of CYP genes using human hepatocytes have been proposed by regulatory authorities. Induction potential of a NCE is assessed initially from the fold increase in mRNA expression of the induced gene. The clinical and regulatory contexts of DDIs predicted from IV-IVE will be discussed.

311. Application of physiologically based pharmacokinetic (PBPK) modelling for the prediction of pharmacokinetic drugdrug interactions (PK-DDIs) and clinical PK-DDI study design

Thomas M Polasek1,2 . Centre for Medicine Use and Safety, Monash University1 , Melbourne, VIC, Australia; CMAX Clinical Research2 , Adelaide, SA, Australia.

Assessing the pharmacokinetic drug-drug interactions (PK-DDIs) of small molecule new chemical entities (NCEs) is an essential part of drug development. If in vitro screening studies identify potential PK-DDIs then in-vitro to in vivo extrapolation (IV-IVE) with static models can stratify risks. Sponsors may then investigate PK-DDIs more thoroughly with physiologically based pharmacokinetic (PBPK) modelling, providing further insights into mechanisms, potential clinical significance, and the need for clinical PK-DDI studies to support regulatory submissions. Although whole-body PBPK modelling started in the 1960s, and its theory dates to a 1937 paper by Teorell, commercial software from the early 2000s made PBPK approaches more accessible to drug developers. Popular software includes Simulations Plus, PK-Sim, GastroPlus and Simcyp – validated drug profiles for studying PK-DDIs are provided. Three groups of data are required for PBPK modelling. Drug specific parameters for perpetrator and victim drugs (e.g., physicochemical properties, in vitro metabolism and transport data, known clinical PK data etc.), systems parameters that describe the molecular and physiological characteristics of a population (healthy volunteers in most DDI studies), and study design parameters, such as number of subjects, doses, durations of treatment, PK sampling times etc. Reversible inhibition, mechanism-based inhibition, and induction of cytochrome P450 (CYP) enzymes are routinely studied with the highest levels of confidence in predicted PK. Interactions with transporters (P-gp, OATP1B1 etc.) and non-CYP enzymes (UGTs, FMOs, SGTs) are less commonly applied by industry, although the quality of these predictions has improved in recent years. Combinations of interaction mechanisms across multiple enzymes and transports are particularly challenging to study but are technically possible with PBPK. Simulations vary depending on the type of PK-DDIs being investigated. Importantly, PBPK modelling is used widely to design clinical PK-DDI studies prior to their conduct. There are also many examples of NCEs for which PBPK predictions replaced clinical data entirely to support dosing recommendations with concomitant drugs in drug labelling. Regulatory agencies strongly encourage PBPK modelling for the prediction of PK-DDIs, and routinely publish scientific papers and contribute to meetings and workshops on the topic.

312. Pharmacoepidemiology approaches for evaluating pharmacokinetic drug-drug interactions in populations

Juliana de Oliveira Costa, Medicines Intelligence Research Program, School of Population Health, UNSW Sydney, Sydney, NSW, Australia.

Identifying and reducing the potential for drug-drug interactions (DDIs) is critical for the clinical management of health conditions given DDIs may reduce or enhance the effects of medicines. DDIs and their impact on population health are expected to further increase in the context of an ageing population using multiple medicines to manage comorbidities. Observational research is essential for estimating the frequency of DDIs in populations, identifying the populations at risk of DDIs, and quantifying the health effects of DDIs. As will be described in this presentation, multiple tools can be used for investigating DDIs at the population-level. These tools range from accessing data sources and compendiums of DDIs to using different types of study designs for estimating populations at risk of DDIs or estimating their health effects. In particular, this presentation will introduce the main sources of DDI information for population-level research and key principles of pharmacoepidemiologic study designs in DDI research, including a brief overview of study designs for estimating health effects of pharmacokinetic DDIs, main sources of bias and how to minimise these biases.

313. Clinical decision support in prescribing systems to mitigate risks from pharmacokinetic drug-drug interactions

Matthew P. Doogue, Discipline of Medicine, University of Otago, Christchurch and Department of Clinical Pharmacology, Te Whatu Ora, New Zealand.

Drug-drug interactions (DDIs) both benefit and harm patients and are a common cause of prescribing errors. Clinical resources for DDIs are in two main formats. Firstly as prescriber guidance, such as drug formularies with information attached to each medicine. Secondly as Clinical Decision Support (CDS) systems that alert prescribers when prescribing. Most current prescribing software incorporates CDS systems based on DDI data sourced from a few suppliers. These provide information on pharmacokinetic interactions between drug pairs taken from product information and primary literature. The same information is provided irrespective of number of medicines, dose regimens, prescriber experience etc. Pharmacodynamic DDIs in CDS resources are limited to a few specific types (e.g. QT prolonging). In hospital practice, polypharmacy is the norm and the number of potential DDIs for any individual patient is high. The consequence of alerting using drug name as the only variable is false positive alerts. The alert information provided by CDS is usually not relevant to the prescribing decision being made. Potentially important alerts are missed because of alert fatigue and potentially important DDIs are not alerted. In the prescribing software used in Canterbury hospitals the vendor DDI CDS is deactivated because of the high rate of false positive alerts and the high alert burden. In Canterbury a small set of local rules are used to alert prescribers to selected DDI risks. For CDS to be effective additional parameters such as dose regimen, medication status (new or ongoing), and clinical context (e.g., specialty clinic vs. acute admission) must be integrated. Furthermore, CDS has an educational role, tailoring the alerts according to a prescriber's experience level could both improve utility and contribute to training. With the rapid evolution of software tools the cost of producing clinical information resources is decreasing. Rigorous processes are needed to evaluate the CDS tools for validity, reliability and utility. In recognition of the risks software as a medical device is coming under greater regulatory oversight. This is an opportunity to standardise CDS requirements to get the right information, in the right place, at the right time to reduce the risks arising from drug drug interactions.

314. Paediatric pharmacoepidemiology: starting to come of age

Madlen Gazarian, MBBS(Hons1), MSc(Clin Epi), FRACP. Consultant in Clinical Pharmacology and Therapeutics, Pharmacoepidemiology and Pharmacovigilance; Specialist Paediatrician; Honorary Associate Professor, Faculty of Medicine and Health, University of New South Wales, Sydney, Australia

Australia's National Medicines Policy aims to ensure all Australians have equitable and timely access to medicines meeting required standards of quality, safety and efficacy and that medicines are used optimally and judiciously. Evidence-based decision-making is one of its core principles, a concept with global relevance. Yet this is challenging to achieve in the paediatric population routinely. Since children are often excluded from clinical trials, the medicines used in routine practice are generally not well supported by high quality evidence derived from the paediatric population, with potential negative consequences for health and economic outcomes for this vulnerable group. Even where clinical trials exist, many paediatric trials are only small in size and short in duration, making it difficult to know about uncommon or rare but potentially serious effects and important longer-term outcomes, which may impact treatment decisions if known. Trials may not have included patients from across the age range (0-18 years) nor assessed clinically meaningful outcomes (of effectiveness, safety, comparative effectiveness/safety) relevant to different age sub-groups, so relevant benefit: risk estimates may not be available for the whole paediatric population. Pharmacoepidemiologic studies offer great opportunity to supplement information derived from clinical trials to help address some of these knowledge gaps and evidence needs, helping to transform judicious decision-making for this vulnerable population. This session will explore the unique needs, challenges, opportunities, and significant recent developments in the field of paediatric pharmacoepidemiology. These will be discussed within the broader context of major paediatric medicines research and regulatory initiatives internationally and explore opportunities for meaningful linkages with rapidly expanding general developments in pharmacoepidemiology research methodologies, data infrastructure, and capabilities, with the powerful potential to transform paediatric medicines use and outcomes in future.

315. Perinatal pharmacoepidemiology

Dr Erin Kelty, Research Fellow & NHMRC Emerging Leader, The University of Western Australia

Pregnant women have traditionally been excluded from clinical research into medication safety, owing to the potential for harm. While this was developed to protect women and their developing fetus, it has not had the desired effect. In contrast, women often avoid the use of medications at the detriment of their own health or are required to take medications with unknown risk. Pharmacoepidemiology has begun to bridge this substantial gap in our understanding of medication safety, using routinely collected health data. This approach has a number of advantages, including being associated with negligible risk, being cost effectives, allowing for longitudinal case ascertainment and follow-up, and examination of medication in a real-world setting. However, careful consideration is required in terms of defining cohorts, comparison groups, exposures, and outcomes to ensure the results are robust and meaningful. Examination of medication safety in pregnancy is essential for ensure pregnant women have equitable access to safe medication, and ensuring the best outcomes for women and their children.

316. Pharmacoepidemiology in adult populations: a focus on prescription opioids

Associate Professor Natasa Gisev, Scientia Associate Professor, UNSW Sydney

Escalating opioid use, misuse and harms is a global health problem. Over the last thirty years, rates of opioid use and harms have been increasing in many high-income countries, including Australia. Approximately 3 million Australians use an opioid each year and Australia is currently ranked 8 th in the highest opioid-using nations. In light of this, there is a need to establish population-based Australian evidence on the long-term drivers and outcomes of prescribed opioid use, including the risk of developing dependence, overdose, and other harms. Using prescription opioids and the Australian adult population as an example, the role of pharmacoepidemiology in generating evidence on the patterns and outcomes of opioid use to support national responses aimed at improving opioid use and reducing opioid-related harms will be discussed.

317. Pharmacoepidemiology with older adults: opportunities and challenges

Ilsa Rose Wojt1 . School of Pharmacy, Faculty of Medicine and Health, University of Sydney1 , Sydney, NSW, Australia.

Introduction. Ageing is strongly associated with multimorbidity and subsequent polypharmacy, yet older adults are frequently under-represented in studies that establish evidence-based guidelines for medication use. Pharmacoepidemiology and toxicoepidemiology can provide real-world data on medication and substance use in older adults that informs on safety, usage and efficacy. This information can be used to guide practice and improve older adults' medication management and quality of life. Aims. This presentation primarily aims to discuss the ways in which pharmacoepidemiology can inform medication use in older adults, the barriers that this field faces and future directions that may be pursued. Discussion. Studies of older adults pose distinct challenges due to various issues including confounding, exclusion, multimorbidity, polypharmacy, frailty, geriatric syndromes and cognitive impairment. Collectively, issues such as reduced sample sizes, can serve as an opportunity to encourage data linkage, cross-collaboration and patient involvement. This presentation will further discuss the formation of questions, methodological considerations and the application and impact of pharmacoepidemiological research. Conclusion. Pharmacoepidemiological studies help fill a post-marketing gap for older adults by informing on medication safety and efficacy which can uniquely help inform policy and practice. Challenges addressed in this presentation can best be addressed through further collaboration and coordinated initiatives within the healthcare sector, both nationally and internationally.

318. A LASEREDD Focus: Lentiviral-Driven Directed Evolution of Optimised G Protein-Coupled Receptors for Biologics Discovery

Christopher Draper-Joyce1 , Saund Yadanar1 , Yiling Yu1 , Zoe Bell1 , Eddy Yang1, Lisa Williams1 , Riley Cridge1 , Ross Bathgate1 , Daniel J Scott1 . The Florey Institute 1 , Parkville, VIC, Australia

G protein-coupled receptors (GPCRs) are the largest class of drug targets, yet identifying and developing selective GPCR-targeting drugs is challenging. Antibodies are emerging as an superior modalities to achieve selective GPCR modulation compared to small molecules. Class A GPCRs comprise the largest number of drug targets, but present several challenges for novel biologics discovery, including: low expression levels; protein instability; limited extracellular accessible epitopes and the need to present the entire receptor as an intact antigen (i.e. not just a soluble ectodomain). Further, generating monoclonal antibodies that exhibit pharmacological effects (agonism/antagonism) at GPCRs has proved difficult, especially given their limited solvent exposed and often cryptic drug pockets. We have developed a novel and superior protein engineering & antibody discovery platform called Lentiviral-Assisted Selection Enabling Receptor Engineering and Drug Discovery (LASEREDD®) to circumvent these challenges. The application of LASEREDD® to several challenging GPCR targets will be presented, along with the use of LASEREDD®-optimised GPCRs for nanobody discovery campaigns. Overall, the LASEREDD® approach promises to overcome the significant barriers that have hindered biologics discovery and optimisation at GPCR targets, paving the way for more efficient & effective discovery of GPCR biologics.

319. Developing a new pre-clinical model of metabolic syndrome for testing pharmacotherapies and beyond.

Maria Jelinic[1], Vivian Tran[1], Holly Brettle[1], Abdulsatar Jamal[1], Henry Diep[1], Hayder Al-Aubaidy[1], Thiruma V Arumugam[1], Christopher G Sobey[1], Antony Vinh[1], Grant R Drummond[1].
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Introduction. Metabolic syndrome (MetS) is a cluster of metabolic disturbances (i.e. hypertension, obesity, dyslipidemia, hyperglycemia and hyperinsulinemia) that affects 1 in 4 people. There are major sexual dimorphisms in the development and consequences of these metabolic disturbances, the mechanisms of which are poorly understood. This is at least partly due to a lack of reliable animal models. As female rodents are highly resistant to diet-induced and genetic metabolic disturbances, an overwhelming majority of studies are in males only.
Aims. To develop a robust diet-induced mouse model of MetS that accurately reflects the clinical presentation of MetS in both sexes and test new therapeutic interventions.
Methods. Six-week-old male and female C57BL/6 mice were fed either a high-fat diet (43% kcal) with high sugar and salt in their drinking water (10% high fructose corn syrup and 0.9% NaCl; HFSS), or normal control diet (NCD) for 10 weeks. At end point, blood immune cell populations were characterized using flow cytometry.

Results. Mice fed a HFSS diet displayed MetS by the completion of the diet regimen, regardless of sex (all NCD vs HFD, respectively; n = 12; $P < 0.05$). Cumulative weight gain (11 ± 0.5 vs. 19 ± 0.9 g in males; 8 ± 0.7 vs. 13 ± 0.9 g in females), blood cholesterol (128 ± 4 vs. 174 ± 9 mg/dL in males; 100 ± 3 vs. 134 ± 5 mg/dL in females), fasting blood glucose (8 ± 0.3 vs. 11 ± 0.3 mmol/L in males; 8 ± 0.1 vs. 10 ± 0.2 mmol/L in females) and systolic blood pressure (121 ± 3 vs. 130 ± 3 mmHg in males; 120 ± 1 vs. 129 ± 3 mmHg in females) were all increased in HFSS-fed mice. In HFSS-fed males, B cells (B220+) were increased in the blood (1070 ± 260 vs 391 ± 98 cells/μl; n=7; P<0.05) compared to NCD mice. Strikingly, females were completely protected from these increases in B cells.
Discussion. These findings highlight B cells as potential drivers of renal inflammation in males, but not females with MetS. The new HFSS diet-induced model of MetS is a robust tool for the preclinical testing of potential therapies for MetS. We have recently used this model to test the therapeutic potential of citrus bioflavonoids and intermittent fasting in MetS.

320. Ensuring sustainability in healthcare through partnering with stakeholders to guide safe and effective medicine use

Dr Mouna Sawan - Early career NHMRC Dementia Centre Research Collaboration (DCRC) Research Fellow in medication management and dementia at the School of Pharmacy, Faculty of Medicine and Health, University of Sydney

Medications are the most common intervention used in healthcare to treat and manage disease, especially in older adults with chronic diseases, including dementia. Unsafe medication practices among older adults with dementia are a leading cause of preventable medication-related harm in Australia and globally. For people with dementia, hospitalisation is associated with further exposure to medication-related harm, resulting in re-hospitalisation and overtreatment and increasing the burden on the healthcare system to unsustainable levels. My research program is focused on placing the individual and their carer at the centre of healthcare. I have established genuine partnerships with national consumer organisations, professional societies, government, and industry members to create, develop and test sustainable solutions across care settings to improve the safe use of medications and reduce avoidable medication-related harm. Examples of outcomes arising from my program of work include the development of a tool to evaluate the organisational culture in aged care and codesigning resources to support people with dementia and their carers in medication management across care settings. My research has been used by the Australian Government Department of Health and the Aged Care New Zealand Health Quality & Safety Commission.

321. Cystic fibrosis transmembrane conductance regulator modulators Elexacaftor/Tezacaftor/Ivacaftor during pregnancy

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Introduction/Aim: Highly effective modulator therapies (HEMT) such as elexacaftor-tezacaftor-ivacaftor (ETI), have enabled more women with CF having babies. However, safety of maternal ETI, especially on developing organs of the fetus, is unknown. We aim to investigate maternal transfer of maternally administered ETI in pre- and postnatal wildtype and F508del-CFTR rats and their bioaccumulation into the developing lungs, gut and pancreas.
Methods:
1) ETI permeability: Wildtype rats at embryonic day (E) 19 and adult rats were administered an intraperitoneal injection of ETI (40mg/kg/d ivacaftor + 6.7mg/kg/d/elexacaftor + 3.5mg/kg/d tezacaftor) traced with [3H] ivacaftor. [3H] radioactivity was measured using liquid scintillation counting.
2) Placental and Milk Transfer: CF and wildtype pups were exposed via placental or milk transfer from dams orally treated with ETI for 6 days. ETI concentrations in pup & maternal plasma were determined by liquid chromatography–mass spectrometry. 3) Histological assessments using H&E staining of tissues were completed and untreated vs treated as well as heterozygous vs homozygous vs wildtype were compared (n=2).
Results: 1)Concentrations of ETI were higher in liver, lung and pancreas; brain ratios remained low (Figure). 2)ETI showed a strong preference to CF target tissues (lung, pancreas), contrasting with restricted entry into brain and CSF (non-target tissues). 3)Histological assessment of the small intestine no showed differences in villi length (CF, 0.336 ± 0.0929 mm vs heterozygous, 0.335 ± 0.118 mm) and villi density (CF, 15.4 villi/mm length vs heterozygous, 16.0 villi/mm length).
Conclusion: ETI does not appear to cause structural damage in small intestine villi and pancreas, suggesting the possible safety to breastfed human newborns. Further work is needed to understand the effects on ETI on the fetus during gestation.

322. From Serotonin to Neuropeptides and back: Translational Research in Pharma and Academia

Prof Emeritus Daniel Hoyer, University of Melbourne

I switched fields multiple times as is common in big Pharma and did lead multidisciplinary teams at Novartis and/or in collaboration with academic institutions or Biotechs, researching on Monoamine receptors and transporters: 5-HT receptors (all except 5-HT5 and 5-HT6), adrenoceptors (alpha1,2 and beta1,2), dopamine (D2, D3 and D4), 5-HTT, NET, DAT. Peptide and other receptors: Orexin, somatostatin (sst1-sst5), endothelin, CRF, NPS, NPY1,2, neurokinin NK1, sphingolipid S1P1-5, Glutamate (mGluR5,7), GABAB. Ligand-gated channels and other receptors: 5-HT3, Glutamate (AMPA, Kainate, NMDA), Nicotinic (alpha4beta2, alpha7), GABAA and peripheral benzodiazepine receptors, FMR1 and FMRP. The lecture will illustrate some historical and more recent basic and translational work done in the 5-HT, somatostatin and orexin fields.

400. Neuroimmune mechanisms of stress: Insights from a mouse model

Prof Tomoyuki Furuyashiki, Kobe University – Japanese Pharmacological Society

Stress from adverse and demanding conditions affects cognitive and emotional functions and risks mental illness such as depression. Since the biological basis of stress has remained elusive, therapeutic development targeting stress has not been established. Rodent studies using stress models have elucidated multiple neurobiological consequences of stress, depending on the stress conditions. We have found that acute stress induces dendritic hypertrophy of prefrontal neurons via the dopamine D1 receptor, augmenting stress resilience, whereas chronic stress attenuates prefrontal dopaminergic activity and induces dendritic atrophy of prefrontal neurons through microglia-driven neuroinflammation, leading to behavioral disturbances. In addition, chronic stress mobilizes leukocytes from the bone marrow, synergizing with neuroinflammation to promote behavioral disturbances. These findings have been considered clinically relevant since clinical studies have reported the association between inflammation and depression. Despite the great advancement in understanding the biological basis of stress, various mysteries remain, such as how acute and chronic stress causes different neurobiological consequences, how the individual variability of stress susceptibility emerges, and how multi-organ interactions underlie stress-induced neuropsychiatric dysfunctions. In this talk, I will present our recent studies about neuroimmune mechanisms of stress with rodent stress models and discuss their relevance to therapeutic developments for mental illness.

401. Attenuation of the PI 3-kinase signalling pathway leads to increases in lifespan and health span

Prof Peter Shepherd, University of Auckland

The p110alpha isoform of PI 3-kinase is an enzyme encoded by the PIK3CA gene that is best known for it’s role in mediating the effects of growth factor receptors. It was therefore not surprising to find that mutations in this gene are commonly found in cancers and mosaic overgrowth syndromes (PROS). This lead to a rush to develop selective inhibitors of PI 3-kinase as anti-cancer agents. Unfortunately the use of these is limited by on target toxicity which is most obviously manifested as an increase in circulating glucose. This arises because p110alpha is also essential for insulin signalling. At the moment this limits the use of p110alpha inhibitors although they are used as single agents to treat overgrowth syndromes. Therefore we undertook a long term studying mice to understand the impact of the p110alpha inhibitor alpelisib on lifespan and health span. This found that in fact the regular administration of this alpelisib increases both lifespan and health span, with these effects being most obvious in female mice. This presentation will discuss these results and also how these results relate to genetic models of PI 3-kinase deficiency.

402. Ovarian metabolism as a target to support women's health with age

Dr Lindsay Wu, The University of Sydney

One of the earliest declines with advancing biological age is female reproductive capacity. This has primarily been attributed to a depletion of the ovarian reserve, yet a decline in oocyte quality occurs well before ovarian depletion. Recently, we found that a decline in the redox cofactor nicotinamide adenine dinucleotide (NAD+) is a reversible cause of declining oocyte quality and female infertility during reproductive ageing, and showed that the NAD+ repletion using metabolic precursors could restore functional fertility with age, resulting in an ongoing clinical trial at the Royal Women's Hospital in Sydney. Here, we show that this strategy can ameliorate accelerated ovarian ageing due to clinically relevant chemotherapy treatment, with impacts on late-life health related to ovarian function including protection against a striking loss of bone integrity. Finally, the clinical development of these NAD+ precursors is complicated by PK/PD studies being confounded by background levels of naturally occurring metabolites, whose levels change in unexpected ways during treatment. We have developed stable isotope tracing strategies to identify unexpected aspects of NAD+ metabolism, and present new insights from this work.

403. Cardiac adiposity in aging; a potential target for treatment of cardiac arrhythmias?

James R. Bell Centre for Cardiovascular Biology & Disease Research & Department of Microbiology, Anatomy, Physiology and Pharmacology, School of Agriculture, Biomedicine and Environment, La Trobe University, VIC 3086.

Visceral adipose depots are highly active endocrine organs, releasing factors into the blood that can deleteriously influence other organs. Given its immediate proximity to the heart, interest in pericardial visceral adipose as a determinant of cardiac risk has rapidly emerged. Pericardial adipose tissue describes the combined epicardial and paracardial adipose depots surrounding the heart. It has important physiological functions in regulating myocardial fatty acid availability, but excess adiposity is a cardiovascular liability. Indeed, cardiac adiposity increases markedly with aging, in obesity, and in post-menopausal women – all significant risk factors for cardiovascular disease. A focus of our lab has been to investigate the strong link between cardiac adiposity and atrial fibrillation. Studies show that both infiltration of adipose into the epicardial surface of the atria and release of pro-fibrotic paracrine factors exert a physical barrier to normal atrial conduction pathways. This leads to regional areas of slowed conduction across cardiomyocytes that can disrupt normal conduction pathways and introduce 're-entrant' activity – repetitive circular electrical pathways that are critical to the onset and maintenance of atrial fibrillation. Our published findings have moved the field forward in a new direction, demonstrating a novel inter-cellular communication axis within the heart between pericardial adipose and neighbouring cardiomyocytes. We showed that this communication axis conveys the paracrine actions of infiltrating pericardial adipose, causing localised structural and electrical remodelling of adjacent cardiomyocytes and promoting the conduction heterogeneity that can culminate in atrial fibrillation. We now extend these findings to further investigate the molecular mechanisms underlying adipose-cardiomyocyte communication and its role in the development of heart disease in aged and obese populations.

404. Hypertension and ageing – a challenging pharmacological landscape

A/Prof Tracey Gaspari, Monash University

Aging is the primary risk factor that underlies hypertension and many other cardiovascular diseases. As we age, the vasculature undergoes structural and functional alterations that are characterised by changes in endothelial function, increases in wall thickness, reduced vascular compliance and arterial stiffening. These changes are brought about by increases in fibrosis and extracellular matrix (ECM) remodelling and are amplified by hypertension. The molecular mechanisms underlying these processes are complex and still being delineated but it is clear that both chronic inflammation and fibrosis play key roles. The combination of aging and prohypertensive elements, including RAAS activation, oxidative stress and inflammation result in excessive fibrosis which often spreads beyond the vasculature into parenchymal tissue, leading to tissue fibrosis, scarring and eventually end organ damage of the heart, kidney and brain. There are no effective cures for fibrosis, with existing antihypertensives having moderate effects on slowing down disease progression but not stopping it. Our work has identified a novel anti-fibrotic target within the RAAS, the enzyme insulin regulated aminopeptidase (IRAP), also known as the AT_4 receptor with pharmacological inhibition of this enzyme shown to not only prevent, but more importantly reverse many of the age-associated changes that occur in the heart, kidney and vasculature in aged mice. Our research is now focussed on development of novel IRAP inhibitors and elucidation of their multi-faceted mechanisms to address the critical unmet need for effective anti-fibrotic therapies.

405. Can the immune system be targeted to treat hypertension and end organ damage?

Prof Grant Drummond, La Trobe University

It has been more than 50 years since Ebringer and Doyle showed that patients with hypertension have elevated serum antibodies1 , thus providing the earliest clinical evidence that the condition is associated with activation of the immune system. But only in the last decade or so has definitive proof emerged that an intact immune system is essential for hypertension and target organ damage to fully develop. In this presentation I will discuss our recent findings highlighting the NLRP3 inflammasome and its cytokine product interleukin-18 as crucial mediators of T cell activation, inflammation and impaired fluid handling in the kidneys during salt-induced hypertension. I will discuss the implications of these findings for our understanding of how a high salt diet and other environmental insults may contribute to hypertension and what they may mean for future therapies. 1. Ebringer A and Doyle AE. Raised Serum IgG Levels in Hypertension. Br Med J. 1970; 2: 146–148.

406. Air Pollution-induced respiratory complications: what about the heart?

Prof Doan Ngo, The University of Newcastle

The World Health Organisation estimates that >7 million premature deaths/year are attributed to air pollution, with most deaths caused by cardiorespiratory complications. Chronic exposure to air pollution contributes to multiple cardiovascular pathologies, including atherosclerosis, hypertension, and arrhythmias. The composition of particulate matter (PM) in air pollution differs depending on geographical location and source of pollution (E.g. bushfire smoke vs urban traffic-related emissions vs remote geogenic/mining dust). How the composition of different PM that Australians are exposed to affect cardiovascular physiology and lead to disease is also completely unknown. The key challenge for prevention and management of these adverse effects is our lack of understanding of how exactly the damage is done and what proteins in the body are critical to these processes. I will address the above knowledge gap of identifying key medicators and regulators of heart damage caused by air pollution particulate matter.

407. The liver-lung axis contributes to secondary bacterial pneumonia

Steven Bozinovski, RMIT University

Respiratory related diseases are a major cause of death and morbidity worldwide. Respiratory viruses and bacterial pathogens that infect the lungs can cause acute lung injury and pneumonia with devastating consequences in susceptible populations. The interaction between respiratory viruses and bacteria is particularly challenging as this can lead to life-threatening secondary bacterial pneumonia (SBP). Improving the efficacy and availability of new vaccines are central to the fight against lower respiratory infections. Equally, there is an urgent need to develop new therapies to reduce lung immunopathology and lung injury during an acute infection. We have established a SBP model in mice and demonstrated that the lungs become infiltrated with a distinct neutrophil/MDSC subset defined by hypersegmentation of the nucleus, reduced CD62L expression and increased iNOS/Arg1 expression, which is consistent with a mature immunosuppressed phenotype. We also show that the liver is producing excessive amounts of the acute phase reactant called serum amyloid A (SAA) during SBP. We identify the molecular axis by which excessive SAA is produced and provide new insight into how this response is maintaining a dysfunctional immunosuppressed neutrophil/MDSC population in the lungs, which fails to eradicate bacterial pathogens underpinning SBP. Our discoveries aim to stimulate new research into blocking liver products as a novel approach strategy to reducing immunopathology and acute lung injury during SBP.

408. They are not born equal

Hui Chen, MD PhD School of Life Sciences, Faculty of Science, University of Technology Sydney, NSW, Australia

There is no safe level of air pollution for human health. In Australia, our biggest threat comes from traffic-related particulate matter (PM), particularly in urban areas and alongside major traffic corridors. PM2.5 is of particularly high risk to human health, and the hazards are unevenly distributed among the general population, with the unborn child particularly vulnerable. Boys' cognitive functions can be significantly reduced by prenatal exposure to PM2.5, whereas girls are less affected. This also applies to other organ systems. While PM has been increasingly recognised as a major in-utero toxin by researchers, the mechanisms of action are not fully understood. In our well established mouse model of maternal exposure to an Australian level of traffic PM, we recapitulated known features of perinatal PM exposure in humans. Male mice have poorer cognitive performance than female littermates. PM is a strong oxidant, which drives adverse health effects. Maternal traffic PM exposure results in oxidative stress and abnormal mitochondrial functional

units in males' brains, not females. RNA sequencing analysis on the newborn brain showed significantly upregulated histone demethylase Kdm6a in female brains which was not changed in male brains. In line with this, PM-females had 23 genes significantly upregulated compared to Sham-females, including those supporting mitochondrial function, cortex and hippocampus formation, synapse growth and maturation, cognitive function, and neuroprotection. In vitro study using differentiated SY-SH5Y neuron cells which are female lineage, showed that upon PM2.5 exposure, Kdm6a was also upregulated. In primary cortical neurons from foetuses, PM exposure suppressed neuron and synaptic numbers, which was prevented by the upregulation of Kdm6a. Oxidative stress induced by PM exposure was also prevented by Kdm6a upregulation. Therefore, timely epigenetic adaptation may play a vital role in female resilience against prenatal PM exposure-induced neurological disorders.

409. Performance of Prescribing Skills Assessment ANZ in final year medical students – student experience and skills performance.

Sarah N Hilmer. Departments of Clinical Pharmacology and Aged Care, Royal North Shore Hospital and Kolling Institute, Northern Sydney Local Health District and The University of Sydney, St Leonards, NSW, Australia.

Introduction. Education in clinical pharmacology, with appropriate prescribing competency assessment, supports medical graduates in making rational prescribing decisions. ANZ medical schools, with ASCEPT, collaborated with the British Pharmacological Society to adapt the Prescribing Skills Assessment (ANZ PSA) for Australasian students as a pilot in 2015. Since then, participation in the exam has increased and now involves the majority of medical schools in ANZ. Aims. To assess student experience and performance in the ANZ PSA from 2017-2019. Methods: A mixed methods approach was applied to student data (n=6440), including student surveys and exam scores. Results. Most students gave positive feedback about the ANZ PSA interface and the clarity of the questions. Approximately one third reported insufficient time to complete the exam and over two thirds reported that they were not well prepared for the ANZ PSA by their medical school course. The average pass rate was 85-89%, and did not differ substantially over time, between different domains tested or between schools. Discussion. The ANZ PSA is well accepted by medical students, although they felt felt rushed and under-prepared. The collaboration through ASCEPT across medical schools in ANZ has formed the foundation for ongoing work together to implement and sustain the ANZ PSA. The goal is to assess prescribing competency consistently and rigorously, initially in all medical graduates and subsquently in all prescribers. Harrison C and Hilmer S (2019) Australian Prescriber, 42(5): 148-150 Chin PKL, Charles K, Murnion B et al (2023) British Journal of Clinical Pharmacology 89(10):3105-3115

410. Preparation for prescribing and Prescribing Skills Assessment in NZ - pre-intern level prescribing, requirements for basic physician training, and beyond

Dr Paul Chin, The University of Otago

A key aspect of educating future prescribers is practice with the prescribing platforms that they will encounter in the workplace. At Christchurch, medical students have had the opportunity for ~10 years to use the inpatient electronic prescribing and administration software in the live setting of acute hospital wards for actual patients, under supervision by clinical teams and with monitoring of activity by the Department of Clinical Pharmacology. This has been accompanied by small group tutorials for medical students that employ the use of a ‘mirror’ prescribing system to use on virtual patients. The use at Christchurch over the last 3 years by final year medical students of a) the ‘mirror’ prescribing system for training and b) the live system in the wards will be described.

411. Preparation for prescribing and Prescribing Skills Assessment at USYD - programmatic approach to skill development, creating simulations for workplace assessments of prescribing, and student feedback about preparation needs

A/Prof Kellie Charles, The University of Sydney

Background and Aims: Final year medical students have consistently expressed a lack of preparedness for prescribing as junior doctors both in Australia and internationally. The emergence of COVID19 challenged pharmacology academics to answer the question: How do we prepare an underprepared final year medical student to complete prescribing tasks in the support our COVID-19 surge medical teams? Summary of work and outcome: To design the new teaching model and assessment, we used design thinking to the develop of a series of complex, clinically-authentic scenarios with matching assessment. In April 2020, we implemented the first workplace-based assessment (WBA) with structured debrief model to 250 students across 7 clinical schools in Sydney and 3 regional clinical schools. The curriculum and assessment

development were informed by self-regulated learning, social situated learning, constructivism and novice-mastery educational theories to ensure the learning in theoretical space could be translated into the clinical ward-based learning. The new model of prescribing teaching and assessment were evaluated using a mixed methods approach and informed by a realist evaluation framework. Discussion: Final year students perceived the tasks were beneficial for learning (median 3.5-4/5 in 2020, 2021). Further in-depth realist interviews were conducted with 2022 cohort students (n=11) at 3 time points in the final year – prior to WBA, after WBA teaching and after final clinical pre-internship training. In the context of feeling very underprepared and nervous about their role in prescribing, the WBAs provided pre-intern students with a gradual exposure to the complexity of prescribing, practical knowledge and skills, familiarisation and practice of relevant prescribing resources and provision of critical and constructive feedback through a structured debrief of experts (mechanisms) to enhance their competence and confidence with prescribing (outcome).
Having a structured series of interview with an educator to review and reflect on the process of learning how to prescribe, discuss potential challenges and opportunities to further learning was an unexpected mechanism for improved recognition and ownership of their learning to develop the essential skills and knowledge needed for prescribing (outcome). Conclusion: Using a design-theory framework allowed the team to incorporate more theoretical approaches into curriculum development as well as promoting iterative educational design. The use of a formal realist evaluation approach enabled a meaningful analysis of the how and why rather than does it work with the research findings will continue to be integrated into the cycles of future iterations.

412. The National Prescribing Curriculum post National Prescribing Service - Where to next?

Dr Ivan Bindoff, Senior Research Fellow, University of Tasmania

The National Prescribing Curriculum (NPC) is a highly valued resource for new and aspiring prescribers from various health disciplines. It is a strongly credentialed, robust and proven solution, with a high degree of engagement across the sector. It is, however, a dated solution, with over 10 years of development history. The University of Tasmania are pleased to present a summary of our 3-year Roadmap for the NPC, which aims to refresh and renew the technology and ensure the clinical content remains contemporary and up to date, while also ensuring everything that has made the program successful remains intact.

413. Model informed drug development (MIDD) to accelerate Phase 3 start

Chiara Zecchin, GSK

Depemokimab is a monoclonal antibody (mAb) that blocks IL5 binding to its receptor and modifies abnormal IL5 biology, including eosinophilic inflammation, thus leading to rapid reduction of blood eosinophil count (BEC). In the first time in human (FTiH) trial (NCT03287310) in mild to moderate asthma, depemokimab showed enhanced potency and extended t1/2 compared to first in class mepolizumab. The aim of the clinical pharmacology team was to expedite clinical development of the second generation long-acting anti-IL5 antibody depemokimab by leveraging MIDD and clinical trial data from first in class mepolizumab. Specifically, to select the Phase 3 dose regimen of depemokimab, in mepolizumab precedented eosinophilic diseases, based on the FTiH data and skip Phase 2 dose-ranging trials. A PKPD model describing mepolizumab inhibitory effect on blood eosinophil was available. The model had been developed on data including various eosinophilic conditions, a wide range of baseline BEC and dosing regimens. The model structure and covariates (disease and baseline BEC) and associated parameters were maintained the same. The PK parameter values, the drug concentration achieving half of the maximum inhibitory effect, the interindividual and residual unexplained variability were estimated using depemokimab FTiH data. Clinical trial simulation using this model identified the depemokimab dose predicted to achieve pharmacology comparable to the mepolizumab approved dose, in the same disease. This dose was considered the optimal phase 3 dose. The use of MIDD and pharmacology principles, the accumulated knowledge on safety and efficacy of neutralisation of the IL5 pathway, and of the pharmacology-clinical efficacy relationship, provided a strong rationale to progress depemokimab from FTiH directly to pivotal Phase 3 trials in four indications. MIDD was key in supporting the dose rationale during regulatory interactions.

414. Ethnopharmacology: past, present and future

Romina A Nand1 , Carwyn Davies1 , Ying Ke1 , Frances V Stringer1 . Clinical Pharmacology Modelling & Simulation1 , GSK R&D, Abbotsford, VIC, Australia.

Ethnopharmacology in drug development is the evaluation of potential inter-ethnic differences in exposure or response to medicines, which may arise due to genetic variations, environmental factors, or cultural practices. Ethnicity may therefore be one factor to consider when tailoring drug doses to individual patients. In some instances, clinically meaningful inter-ethnic differences in drug response have translated into population specific prescribing recommendations [1]. The evaluation of inter-ethnic differences in drug response was harmonized with the introduction of the ICH E5 (1998) guideline [2] which provides a framework for the consideration of intrinsic and extrinsic ethnic factors in drug development. This assessment is critical for global drug development and facilitates the approval of medicines that are safe and effective for diverse ethnic populations. Ethnic perspectives need to be addressed early during global drug development as recommended in the ICH E17 guideline for multi-regional clinical trials [3]. The US FDA is also promoting enrolment of diverse patient populations in pivotal clinical trials to support drug approval and recommends considering the plan as early as practicable in clinical development for a given indication. Appropriate decisions concerning drug development strategies, ethnic specific study designs and use of relevant tools contribute to a better understanding of whether ethnicity is an important contributor to variation in drug response. This session will describe the journey of Ethnopharmacology in drug development with a goal of understanding potential sources of variability in drug response in diverse ethnic populations. Examples will be presented to highlight the importance of ethnic perspectives in global drug development, its implications for the approval of medicines in countries with multi-ethnic populations or in Asian markets, and how these have evolved with time. [1] Ramamoorthy A et al (2022) J Clin Pharmacol. 62: 486-493. [2] ICH Guideline: Ethnic Factors in the acceptability of foreign clinical data (E5, R1). [3] ICH Guideline: General principles on planning and designing of multi-regional clinical trials (E17).

415. Impact of Clinical Pharmacology in Paediatric Drug Development

Shaun S Kumar1 . Clinical Pharmacology, Modeling, and Simulation, Parexel International, North Ryde, NSW, Australia.

Historically, the paediatric population has been overlooked in the drug development process whereby little to no data were collected until drugs were approved. The legal framework governing paediatric research has undergone significant changes to ensure the safety and effectiveness of medications for children. The Best Pharmaceuticals for Children Act (BPCA, 2002) and the Pediatric Research Equity Act (PREA, 2003) in the US are designed to incentivise and/or mandate biopharmaceutical companies to conduct pediatric studies. In 2022 the US Food and Drug Administration (FDA) released the draft guidance titled “General Clinical Pharmacology Considerations for Pediatric Studies of Drugs, Including Biological Products.” This document provides guidelines for the design, implementation, and analysis of pediatric studies. It emphasizes the importance of considering developmental changes in PK, PD, and PG during pediatric drug development. Several maturation processes are now understood that describe developmental changes in PK which include weight-based allometry, CYP enzyme ontogeny, and renal function maturation. Furthermore, the design of paediatric clinical trials needs additional considerations similar to those in adult populations. For example, determination of the optimal dose and timing and the number of PK/PD samples to be collected are important considerations. This presentation will provide a brief overview of the current regulatory framework for paediatric drug development, an overview of the FDA guidance document, a description of weight-based allometry, CYP enzyme ontogeny, renal function maturation, and trial design considerations.

416. Leveraging Model-Informed Drug Development (MIDD) in Oncology: A Case Study with Relugolix

Dr Yu-Wei Lin, Monash University / Certara

Model-informed drug development (MIDD) is unarguably the cornerstone of pharmacological research in the 21st century. It refers to the strategic creation and integration of mathematical models with throughout plan of execution (key questions, assumption, modelling approach and documentation) to facilitate the decision-making process in pharmaceutical research. The applications of MIDD range from novel target identification, formulation design, non-clinical and clinical development and biopharmaceutical research to trial design and cost-effectiveness evaluations. MIDD is also increasingly used to evaluate causal links between drug physiochemical properties, disease/pathogen biology and patient physiology. This has facilitated an integrated approach for effective trial designs and data-to-knowledge transformation while also helping narrow knowledge gaps and maximise the therapeutic potential of drug candidates.

This presentation will use relugolix, the first orally-active, non-peptide gonadotropin-releasing hormone (GnRH) receptor antagonist, as a case study to demonstrate the significance of MIDD in modern drug development programs.

417. Human duodenum uptake and animal model toxicity of oral insulin nanotechnology (EA-1)

Nick Hunt[1,2], Glen Lockwood[1,2], Scott Heffernan[2], Meng Ng[2], Lara Westwood[1], David Le Couteur[2], Victoria Cogger[1,2]. [1]Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia; [2]Sydney Local Health District, NSW Health, Sydney, NSW, Australia.

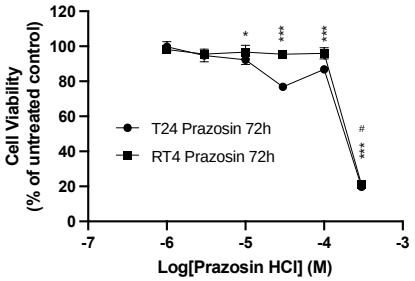
Injectable insulin is an extensively used medication with potential life-threatening hypoglycaemic events. Previously we presented the formulation of a silver sulfide quantum dots (QDs) with a chitosan/glucose (CS/GS) encapsulating polymer to produce an oral insulin nanocarrier (QD-INS-CS/GS) (1). Here we examined the uptake of this oral insulin formulation in human duodenal tissue explants and examined chronic dosage toxicity in three animal models (mice, rats and baboons). We have demonstrated our oral formulation increases absorption in human duodenum explants by 40-fold compared to regular insulin alone.

Mice were treated 3x in a week, with vehicle, high and very high dosages of QD-INS-CS/GS (n=3 per group). These formulations contained QDs at 0, 100, 300 ug/kg and insulin at 0, 100, 300 IU/kg. Analysis of biochemistry and lipid data showed no effects of high or very high oral insulin treatment on circulating albumin, amylase, bilirubin, creatinine, protein, yGT, ALP, ALT, AST, cholesterol, triglycerides, HDL or LDL compared to control mice. Streptozotocin treated (65 mg/kg; single dose) rats were treated with QD-INS-CS/GS containing QD at 150 ug/kg/day and insulin at 150 IU/kg/day (n=5 per group) for 6 weeks via drinking water. Control rats received subcutaneously injected insulin (40 IU/kg/day). No changes in the above blood tests were reported, QD-INS-CS/GS did show a reduced ALT compared to injected insulin (100 U/L vs 150 U/L p = 0.0498). Non-diabetic baboons (n=20) were treated with escalating dosages of QD-INS-CS/GS. Dosages were 25, 50, 125, 250 IU insulin containing QDs at 25, 50, 125, 250 ug. Baboons received one treatment a month for 4 months. Following treatment, no biochemical or haematological toxicity, or adverse events (hypoglycaemia) were observed. These studies demonstrate the successful application of this oral insulin platform with human in vitro tissue and the safety of this technology platform.

(1) Hunt N et al (2023) Preprint https://www.researchsquare.com/article/rs-2440528/v1

418. Assessing alpha-1 antagonist prazosin cytotoxicity on bladder cancer cells and bladder function

Liam A O'Callaghan[1], Russ Chess-Williams[1], Katie Powell[1], Catherine McDermott[1]. Faculty of Health Sciences and Medicine, Bond University[1], Gold Coast, QLD, Australia.



Introduction. Bladder cancer is a common, complex disease. Current treatments involve intravesical chemo- or immuno-therapy but are hindered by frequent recurrence and local side effects that impact quality of life.
Aims. The aims of this study were to evaluate the cytotoxicity of the α1-ADR antagonist prazosin in bladder cancer cell lines, as well as explore the effects of luminal prazosin treatment on bladder function using a porcine model.
Methods. T24 and RT4 cells were treated with prazosin (1-300 µM) or vehicle (DMSO) for a 2-hour period mimicking the duration of intravesical treatment. Cell viability was assessed at 24-72 hours using the resazurin reduction assay. The luminal surface of female porcine bladders were treated with prazosin (300 µM) or vehicle in modified Ussing chambers for 2 hours, followed by organ bath studies to assess functional responses to carbachol, isoprenaline, adenosine triphosphate (ATP), electrical field stimulation (EFS), potassium chloride (KCl) Krebs.
Results. Pre-treatment of T24 and RT4 cells with prazosin resulted in a statistically significant concentration-dependent decrease in cell viability at 24-72 hours (Figure 1). Pre-treatment with 300 µM prazosin did not significantly affect nerve evoked contractile bladder responses. ACh remained the dominant neurotransmitter in control and treated tissues. Responses to purinergic stimulation, relaxation to isoprenaline and general bladder contractility were similarly unchanged. However, the maximal response of full-thickness bladder tissues to carbachol was significantly enhanced by prazosin ($P<0.001$), while responses of denuded detrusor and urothelium/lamina propria to muscarinic stimulation were unchanged.
Discussion. The study elucidates that prazosin exerts a concentration-dependent cytotoxicity on invasive T24 and non-invasive RT4 bladder cancer cells, thereby presenting a promising avenue for targeted therapeutic interventions in bladder cancer. Notably, the limited changes to normal bladder function in the porcine model underscores prazosin's potential as an intravesical agent with an advantageous safety profile.

419. A novel mouse model of bladder mucosal denervation

Cindy Tay[1], Stewart Ramsay[2], Vladimir Zagorodnyuk[1], Natalie Stevens[1,3], Andrea Harrington[1,3], Stuart Brierley[3], Luke Grundy[1]. FHMRI, Flinders University, Adelaide, SA, Australia[1]; Adelaide Medical School, The University of Adelaide, Adelaide, SA, Australia[2]; SAHMRI, Adelaide, SA, Australia[3].

Introduction: There are two major types of sensory nerves present in the bladder: 1) stretch-sensitive nerves in the detrusor smooth muscle that transduce bladder stretch into sensations of fullness; and 2) stretch-insensitive mucosal nerves that are found in the lamina propria within and around the urothelium. The physiological role of mucosal sensory nerves in normal bladder function is currently unknown; however, given that they lie near the bladder lumen, we hypothesise that the mucosal afferents are ideally placed to detect bladder damage and inflammation.
Aim: To develop and characterise a mouse model of bladder mucosal denervation.
Methods and Results: Seven days after intravesical infusion of Resiniferatoxin (RTX; 30μM for 30min), an ultrapotent TRPV1 agonist that causes sensory neuron death when used in high concentrations, $Na_v1.8$ and CGRP immunoreactive nerve fibres were significantly reduced in the bladder mucosa but not in the detrusor (N=5; $p<0.001$ and $p<0.0001$ respectively). Utilising *ex-vivo* bladder afferent electrophysiological recording preparations, we found RTX treatment significantly reduced bladder afferent responses to mucosal stroking (N=5; 10mg $p<0.01$, 50mg $p<0.001$, 100mg $p<0.05$) but not muscular stretch (N=5; 1g, 3g, 5g $p>0.05$). The proportion of responsive mucosal afferents per preparation was also significantly decreased (N=5; $p<0.001$). Despite obvious deficiencies in bladder afferent function, mucosal denervation had no impact on bladder function measured using a void spot assay (N=8; $p>0.05$), or sensitivity to von-Frey probing of the abdomen (N=10; $p>0.05$). Immune cell phenotyping of RTX bladders 7 days after treatment using flow-cytometry showed no significant difference in immune cell numbers compared to control bladders (N=8; $p>0.05$).
Discussion: Intravesical infusion of RTX selectively denervates bladder mucosal afferents without inducing bladder inflammation. As voiding function was not affected after RTX treatment, this suggest that mucosal stretch-insensitive afferents are not crucial regulators of normal bladder voiding function. This novel model of bladder mucosal afferent denervation can now be utilised to understand the contribution of mucosal afferents to bladder function in animal models of bladder inflammation.

420. The Functional Role of Phosphodiesterase Isoenzymes in the Isolated Porcine Urethra

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Introduction. Previous research has suggested a role for phosphodiesterase (PDE) isoenzymes in the control of the urethral smooth muscle contractility (Abrams et al., 2010; de Groat & Yoshimura, 2015; Rahardjo et al., 2021).
Aims. The present study aimed to investigate the role of PDE-4 and PDE-5 isoenzymes in the isolated porcine urethral smooth muscle and mucosal layers to identify potential targets for stress urinary incontinence management.
Methods. Using an organ bath setup, the effects of roflumilast (PDE-4) and sildenafil (PDE-5) (0.1 nmol/L – 10 μmol/L) on isolated porcine urethral mucosa-intact smooth muscle, denuded smooth muscle and mucosal layer were investigated. Unpaired Student's *t*-tests or a one-way ANOVA followed by a Dunnett's multiple comparisons tests was performed to identify statistically significant differences. A p-value of < 0.05 was considered statistically significant.
Results. The dose-dependent relaxation by roflumilast in urethral smooth muscle tissue strips with mucosa-intact was significantly enhanced compared to the denuded strips ($p<0.05$). Inversely, the relaxation induced by sildenafil was greater in denuded strips ($p<0.05$). In the presence of nitric oxide (NO) donor sodium nitroprusside (SNP), the attenuation effect of sildenafil in the denuded strips was enhanced ($p<0.05$). Sildenafil, in the presence of SNP, was more potent than roflumilast in attenuating the tonic contractions of the urethral smooth muscle strips (45% vs 24%). In the urethral mucosal strips, roflumilast (10 nmol/L and above) and sildenafil (1 μmol/L) significantly reduced the rate of spontaneous contraction ($p<0.05$).
Discussion. The results from the study suggest a potential role of the cAMP pathway in modulating spontaneous contractions within the mucosa, while the NO / cGMP pathway appears to be important in modulating urethral smooth muscle tonic contractions. Additionally, the findings also suggests that the presence of the mucosa may inhibit endogenous NO production. The complex interplay between the cAMP and cGMP pathways could be further investigated and identified as potential targets for SUI treatments.

Abrams P et al (2010) Neurourol Urodyn 29:213-240
De Groat WC & Yoshimura N (2015) Handb Clin Neurol 130:61-108
Rahardjo et al (2021) Res Rep Urol 13:139-145

421. Effects of cranberry and D-mannose on an *ex-vivo* porcine model of UPEC-induced bladder infection.

Jenane Konesan[1], Kylie Mansfield[2], Lu Liu[1], School of Medical Sciences, UNSW[1], Sydney, NSW, Australia; Graduate School of Medicine, University of Wollongong[2], Wollongong, NSW, Australia

Introduction. Urinary tract infections (UTIs) are common infections primarily caused by *uropathogenic E. coli* (UPEC). These infections are typically treated with antibiotics. However, due to the rise in antimicrobial resistance, there is a demand to identify other nonantibiotic alternatives, including cranberry and D-mannose, which have been assessed in clinical trials for managing UTIs.
Aims. This study aimed to investigate whether cranberry and D-mannose can protect against UPEC-induced damage to the porcine bladder, a model recognised as the most accurate comparative model for human.
Methods. Fresh female porcine bladders were placed in chambers with media separately bathing the luminal side and serosal surface. The luminal side was treated with antibody-free media (control), cranberry (3 mg/mL) or D-mannose (10 mM), with or without UPEC (UTI89, OD600:0.4) for 4 hours at 37°C. Mucosal strips after each treatment were obtained for organ bath experiments to investigate the contractile response to acetylcholine (ACh), neurokinin A (NKA) and 5-HT (n=8). A section of tissue was isolated for examination of cellular damage by H&E staining and immunohistochemistry of uroplakin III.
Results. The concentration-response curves of mucosa strips in response to ACh, NKA or 5-HT-elicited contractions were superimposed in all groups, with no significant changes in EC_{50} values and maximal responses. UPEC-treated porcine bladders exhibited significant urothelial damage and oedema compared to the control ($p < 0.0001$). Neither cranberry nor D-mannose was able to protect against this damage. UPEC treatment also significantly damaged the uroplakin layer ($p = 0.0022$, compared to the control). Again, no protective effect was observed with cranberry or D-mannose in the presence of UTI89.
Discussion. Although some oedema was observed within the submucosal layer, where the contractile mechanisms involve myofibroblasts and thin smooth muscle bundles, the majority of the UPEC-induced damage was confined to the urothelial layer and did not result in noticeable changes in contractility. UPEC caused significant damage to the urothelium and uroplakin layer, and neither cranberry nor D-mannose demonstrated a reduction in this damage. Therefore, the effectiveness of cranberry and D-mannose for UTI treatment may be questionable.

422. Comparison of diabetes-induced bladder dysfunction *in vitro* and *in vivo*

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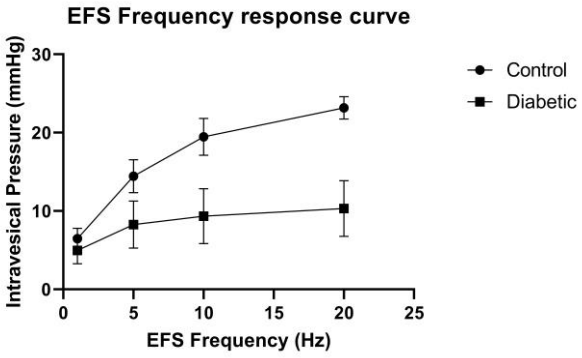
Introduction. Bladder dysfunction affects a majority of patients with diabetes and undergoes a time-dependent change, exacerbated by poor glycaemic control, from the compensated to decompensated state.
Aims. We aimed to use *in vitro* and *in vivo* studies to investigate the mechanisms involved in diabetes-induced bladder dysfunction.
Methods. Diabetes was induced in female mice by treating with streptozotocin (50mg/kg ip daily for 5 days). Bladder function was examined 11 days later using isolated whole bladder preparations (WBP) and urethane-anaesthetised (0.9g/kg sc and 0.3g/kg ip) cystometry.



Results. Streptozotocin treatment increased ($P<0.05$) blood glucose from 9.3±0.4mmol/L (n=14) to 19.1±1.2mmol/L (n=15) and urine output was increased four-fold ($P<0.02$). In isolated whole bladders, increases in intravesical pressure in response to electrical field stimulation were significantly reduced in diabetic mice (see Figure). At 20Hz bladder responses from diabetic mice (10.3±3.6mmHg, n=4) were significantly lower ($P<0.03$) than controls (23.2±1.4mmHg, n=6). Similarly, pressure responses to the primary neurotransmitter adenosine triphosphate (ATP), were reduced ($P<0.04$) in diabetic mice (16.9±3.6mmHg) compared to controls (23.9±0.9mmHg). Also in vivo, cystometric experiments showed the peak pressure during voiding was reduced ($P=0.03$) in diabetic animals (28.16±0.89mmHg, n=11) compared with controls (32.40±1.63mmHg, n=8).
Discussion. The results suggest that short-term diabetes results in a reduced pressure development during voiding, which is caused by depressed bladder neurogenic contractions resulting from reduced bladder responses to the primary neurotransmitter ATP.

423. Testosterone and the transgender heart, Insight from a transgender male mouse model.

K Robertson[1], A Fulton[1], G Gouws[1], L Mahoney[1], B Beck[1], D Donner[2], J Peart[1], E Du Toit[1]. Griffith University[1], Gold Coast, QLD, Australia. Baker Heart and Diabetes Institute[2], Melbourne, VIC, Australia.

Introduction. Masculinising hormone therapy (MHT) is used in female-to-male gender transition (FM-GT) and is associated with increased cardiovascular risk. However, the effects of MHT on cardiac outcomes remain unclear.
Aims. investigate the impact of different MHT dosages on cardiometabolic risk and cardiac tolerance to ischemia/reperfusion injury in mice.
Methods. Fifty-six, 12-week old, C57BL/6J male mice received weekly subcutaneous injections of vehicle (C; n=14), or 9mg/kg (F2M↓; n=14), 18mg/kg (F2M, n=14), or 36mg/kg (F2M↑; n=14) of testosterone (as Sustanon 250) for 14 weeks. We assessed body mass, fasted blood glucose, serum insulin, serum triglycerides, and unfasted serum testosterone and 17β-oestradiol. Isoflurane was used at 4% for induction and 2% to maintain anaesthesia during echocardiography which was performed before and after MHT to assess in-vivo cardiac structure and function. Mice were anaesthetised with sodium pentobarbital (60mg/kg) and hearts excised for Langendorff perfusion to evaluate pre- and post-ischemic cardiac function. Lactate dehydrogenase (LDH) was quantified as a measure of cell death.
Results. MHT induced dose-dependent increases in serum testosterone (C vs F2M, F2M↑; $p<0.0001$) and reduced serum 17β-oestradiol levels (C vs F2M↓, F2M, F2M↑; $p<0.001$). MHT increased total body mass ($p<0.05$) but only altered insulin resistance in animals treated with intermediate dose (C vs F2M; $p<0.05$). Echocardiographic myocardial performance was reduced (C vs F2M, F2M↑; $p<0.05$) and cardiac hypertrophy observed in MHT treated animals (C vs F2M, F2M↑; $p<0.01$). High-dose MHT impaired cardiac tolerance to ischemia/reperfusion injury (C vs F2M↑; $p<0.05$). Analyses of LDH and serum triglyceride data is currently being performed.
Discussion. Outcome sex hormone levels evidence success in modelling of FM-GT in mice. MHT in FM-GT may increase cardiometabolic risk with changes to body weight and glycaemic control but saliently MHT hypertrophied the heart. Testosterone driven myocardial hypertrophy may explain the reduced in-vivo myocardial performance and impaired tolerance to ischaemia/reperfusion observed in this FM-GT animal model. This is the first study to assess ischaemia/reperfusion tolerance in a transgender animal model. We are currently interrogating genetic and proteomic changes in the myocardium caused by MHT and identify mechanistic pathways that govern the results discussed here.

424. Sex matters – impact of HFD and low-dose SGLT2i on rodent cardiometabolic phenotype

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Introduction. Cardioprotection conferred by sodium glucose co-transporter 2 inhibitors (SGLT2i) differs based on sex and comorbidities (Sharma et al., 2023). The mechanisms behind these sex-specific effects remains unknown.
Aims. To investigate the effect of high fat diet (HFD) and the SGLT2i, dapagliflozin, on cardiometabolic phenotype in male and female mice.
Methods. 6-week-old male and female C57BL/6J mice commenced high fat diet (HFD; 60% kJ lipids) or chow. At 18 weeks of age, HFD mice were randomised to 8 weeks of dapagliflozin (target plasma concentration of 122.67 ng/mL) or vehicle (20% Trappsol) treatment via s.c. osmotic mini-pumps (n=8-12 per treatment group). Body weight, glucose and insulin tolerance, left ventricular (LV) systolic function and the plasma metabolome and lipidome were assessed.
Results. At study endpoint, the average plasma concentration of dapagliflozin in male and female HFD mice was 62.3±5.7 ng/mL and 92.8±20.1 ng/mL, respectively. Body weight was elevated in HFD females compared to chow (35.3±2.1 g vs. 28.9±0.9 g, $p<0.01$); dapagliflozin had no effect on body weight. HFD reduced glucose tolerance in females when compared to chow (area under the curve [AUC]: 999.3±114.1 vs. 653.1±58.3, $p<0.05$), while in males, dapagliflozin improved glucose clearance compared to vehicle (AUC: 857.0±66.3 vs. 1399.0±178.0, $p<0.05$). HFD-associated reductions in insulin tolerance in female mice were improved with dapagliflozin treatment (AUC: 614.2±26.5 vs. 777.5±20.5, $p<0.001$). LV systolic function was reduced in HFD males compared to chow (LV ejection fraction: 52.7±2.2% vs. 62.1±2.8%, $p<0.05$), with no effect of dapagliflozin on cardiac function. Although plasma lipid and metabolite profiles were altered with HFD across both sexes, dapagliflozin had minimal effect.
Discussion. The prediabetic phenotype associated with HFD and the metabolic effects of low-dose dapagliflozin differ in male and female mice, highlighting the need for further sex-specific interrogation of the cardiometabolic pathways affected by higher doses of SGLT2i in animal models with established diabetes.

Sharma A et al (2023) Lancet Reg Health West Pac 33:100692

425. Lipoxin A_4 improves cardiac remodelling and function in diabetes-associated cardiac dysfunction.

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Introduction. Chronic inflammation is a key contributor to diabetic heart disease. A crucial aspect of a self-resolving inflammatory response is the production of locally active lipid mediators such as lipoxin A_4 (LXA_4) which initiate and promote the resolution phase[1]. However, the therapeutic potential of LXA_4 in diabetic hearts remains to be explored.
Aim. To investigate the therapeutic effects of adjuvant LXA_4 on diabetes-associated cardiac dysfunction.
Methods. 6-week-old male $ApoE^{-/-}$ mice were injected with vehicle/streptozotocin (55 mg/kg/day i.p. for 5 days) to induce diabetes. After 10 weeks of diabetes, mice were randomly allocated to receive either LXA_4 (5 µg/kg i.p.) or vehicle (0.02% ethanol) twice/week for a further 6 weeks. HbA_{1c} levels, left ventricular (LV) structure, and function were assessed.
Results. Diabetic mice exhibited elevated HbA_{1c} levels, and reduced body weight. Also, the higher macrophage content, elevated M1-like macrophage marker, increased collagen deposition, and upregulated expression of the lipoxin/formyl peptide receptors 2 (FPR2) axis, including (m*Fpr2* and m*Alox15*), were observed in their cardiac tissue. The diastolic dysfunction (e.g. prolonged deceleration time and the isovolumetric relaxation time) was evidenced in diabetic mice. Interestingly, administration of LXA_4 decreased the expression of M1 markers, m*Fpr2*, and m*Alox15*, reduced collagen deposition and improved diastolic function.

	Non-diabetic mice		Diabetic mice	
	Vehicle	LXA_4	Vehicle	LXA_4
Body weight (g)	32.3±0.4 (n=30)	31.3±0.4 (n=20)	25.6±0.7* (n=18)	24.3±0.7* (n=20)
HbA1c (%)	4.6±0.1 (n=19)	4.6±0.1 (n=13)	11.9±0.2* (n=18)	11.8±0.3* (n=20)
Cardiac collagen deposition (%)	1.6 ±0.2 (n=11)	1.4±0.3 (n=11)	3.1±0.4$^{\$\$}$ (n=9)	2.1±0.3$^{\#}$ (n=12)
Macrophage number (NO./0.43mm²)	11.7±1.1 (n=13)	12.4±1.2 (n=12)	18.2±2.6$^{\$\$}$ (n=9)	14.1±1.1 (n=9)
m*S100A9* (fold increase)	1.0±0.4 (n=17)	1.6±0.4 (n=9)	9.2±3.7$^{\$\$}$ (n=12)	2.2±0.6$^{\#\#}$ (n=12)
m*Fpr2* (fold increase)	1.0±0.2 (n=17)	0.8±0.1 (n=9)	4.0±0.9$^{\$\$\$\$}$ (n=12)	2.1±0.4$^{\#}$ (n=12)
A*lox15* (fold increase)	1.0±0.2 (n=17)	1.5±0.6 (n=9)	6.6±2.1$^{\$\$\$}$ (n=12)	3.2±0.8$^{\#}$ (n=12)
Deceleration time (ms)	20.4± 1.1 (n=12)	22.2±0.8 (n=16)	30.5±2.1$^{\$\$\$\$}$ (n=8)	25.6±0.7$^{\#\#\#}$ (n=14)
Isovolumetric relaxation time (ms)	22.9± 0.6 (n=12)	21.6±0.7 (n=16)	26.0±1.0$^{\$\$}$ (n=8)	21.5±0.8$^{\#\#\#}$ (n=14)

*P<0.0001 diabetic vs non-diabetic;$^{\$}$P<0.05, $^{\$\$}$P< 0.01,$^{\$\$\$}$P<0.001, $^{\$\$\$\$}$P<0.0001 vs non-diabetic + vehicle; $^{\#}$P<0.05, $^{\#\#}$P<0.01,$^{\#\#\#}$P<0.01 vs diabetic + vehicle, (2-way ANOVA, Fisher's post-hoc for multiple comparisons).

Discussion. We showed that LXA_4 protects against diabetic heart by reducing adverse remodelling and improving cardiac function. LXA_4-based therapy might be a novel approach to treating diabetic-associated heart disease.

[1]Hodges, R.R., Serhan, C.N., et al. (2017) Mucosal Immunology 10: 46–57.

426. Characterising vascular endothelial cell heterogeneity during angiotensin II-induced hypertension

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Introduction. Hypertension is the leading cause of death, affecting one third of adults worldwide. Aortic stiffening and endothelial dysfunction are hallmarks of hypertension, however the interactions between these two conditions remain largely unknown.
Aims. To characterise endothelial cell (EC) heterogeneity within the mouse aorta and compare EC phenotypes in healthy and hypertensive settings.
Methods. Hypertension was induced by infusing angiotensin (Ang) II (0.7 mg/kg/day) into 12-week-old male C57BL/6 mice via osmotic minipump (*s.c.*). Normotensive control mice received vehicle (saline). After 28 days, mice were killed and aortae were harvested and enzymatically dissociated into single-cell suspensions. Metabolically active live cells were collected using FACS and prepared for single-cell RNA sequencing using Chromium 10x and NovaSeq genomics platforms.
Results. Single-cell transcriptomic analysis of 22,207 cells identified 17 cell types including 2 distinct *Cd31*-expressing EC subclusters in the mouse aorta. Interestingly, von Willebrand factor (vWF), a widely accepted pan-EC marker, was expressed in EC1, but not EC2. Immunofluorescent co-localisation and flow cytometry confirmed the presence of two distinct EC populations. Gene ontology (GO) analysis revealed hypertension enriched for biological processes associated with ECM organisation, cell adhesion and cell migration in EC1, suggesting that a pro-fibrotic and remodelling phenotype. Conversely, GO terms associated with angiogenesis were most prevalent during hypertension in EC2.
Discussion. Identifying distinct aortic EC subtypes may provide novel insights into EC-driven molecular mechanisms of aortic stiffening in the context of hypertension and serve as potential targets of pathological remodeling.

427. Targeting the AT_4 receptor/IRAP reverses type 2 diabetes-induced cardiovascular dysfunction and remodelling.

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Introduction. Cardiovascular diseases (CVDs) are the leading cause of death in patients with type 2 diabetes (T2D). Insulin regulated aminopeptidase (IRAP), also known as the angiotensin type 4 receptor (AT4R), has protective effects in a number of CVD models, however to date the effect in a type 2 diabetic model of CVD has not yet been explored.
Aims. Compare effect of chronic IRAP inhibition on cardio-renal pathology in a rat model of type 2 diabetes (T2D).
Methods. Male Sprague-Dawley rats (n=10/group, 8 weeks old) were placed on a high fat diet (HFD), 2 weeks later rats received 2 x daily injections of low-dose Streptozotocin (35mg/kg ip.) or citrate vehicle. Blood glucose and blood pressure (BP) were measured fortnightly. After 8 weeks of diabetes rats were administered either: vehicle, the novel IRAP inhibitor (IRAPi, 0.72mg/kg/day sc. via mini-pump, implanted under 5% isoflurane inhalation anaesthetic), the SGLT2 inhibitor Dapagliflozin (Dapa, 1mg/kg/day po.) or combination (IRAPi + Dapa) for a further 8 weeks. Cardiac function was assessed before and at end of treatment, following which rats were killed and tissues collected.
Results. No treatment intervention altered BP or glucose handling, although Dapa reduced overall blood glucose levels by ~50%. Diastolic dysfunction was established in all T2D groups after 8 weeks of diabetes (E/A ratio: T2D 1.09±0.04 vs Cit Veh: 1.38±0.10, n=10/group; $p<0.05$). Diastolic function was improved following 8 weeks of IRAPi, Dapa or combination treatment (E/A ratio: Cit Veh: 1.35±0.19; T2D: 1.02±0.15, $p<0.05$ vs Cit Veh; IRAPi: 1.14±0.12; Dapa: 1.20±0.21; Combo: 1.18±0.16. N=10/group). T2D significantly increased cardiac fibrosis (collagen % area 5.4±0.6, n=10) compared to citrate controls (2.9±0.2%, n=10; $P<0.05$). IRAPi and combination treatment reversed fibrosis more effectively than Dapa alone (collagen % area: IRAPi: 3.5±0.3%, $p<0.05$ vs T2D; Combo: 3.1±0.2%, $p<0.05$ vs T2D; Dapa: 4.1±0.4%. N=10/group). Similar protective effects were observed in the kidney and vasculature.
Discussion. IRAP inhibition exhibited cardiovascular and renal protective properties in a rat model of T2D. IRAP inhibition demonstrated better anti-fibrotic action with similar functional benefits when compared to the SGLT2 inhibitor Dapagliflozin. Combination therapy may have additional benefits, including reduction of hyperglycaemia. This study suggests that targeting IRAP provides an effective therapy against T2D-induced cardiovascular end-organ pathologies.

428. Contrasting the pro-oxidant/pro-inflammatory profile of the type 2 diabetic (T2D) rat myocardium with T2D human aortic endothelial cells

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Introduction. Cardiovascular disease incidence is three-fold higher in people with type 2 diabetes (T2D) and accounts for over 50% of mortality in this population. In T2D, inflammation and oxidative stress promote endothelial dysfunction and adverse cardiac remodelling, which are early events in the pathogenesis of cardiovascular disease.
Aims. Compare the inflammatory and oxidant profiles in T2D human aortic endothelial cells (ECs) to those in two preclinical rodent models of T2D to evaluate how closely they mimic changes in these pathways in human T2D.
Methods. Non-diabetic and T2D human aortic ECs were incubated for 48 h in low- (5 mM) or high-glucose (30 mM) conditions. Eight-week-old male Sprague-Dawley rats were placed on a moderate- or high-sucrose high-fat diet (HFD), or remained on standard chow. After two weeks, rats received low-dose streptozotocin (STZ; 35 mg/kg i.p. for two consecutive days), or an equal volume of citrate vehicle. At 22 weeks of age, the left ventricle (LV) was collected. Gene and/or protein expression of markers of inflammation (Vcam-1, vascular cell adhesion molecule 1; Mcp-1, monocyte chemoattractant protein 1; TNF-α, tumour necrosis factor-alpha; p65 subunit of NF-κB, nuclear factor kappa B) and redox stress (Gpx1, glutathione peroxidase 1; $p22^{phox}$) were measured by qRT-PCR and western blotting, respectively.
Results. In T2D human ECs, antioxidant *Gpx1* and pro-inflammatory *Mcp-1* gene expression were higher, regardless of glucose incubation conditions; pro-inflammatory *Vcam-1* was only elevated under high-glucose conditions. In T2D rats, LV gene expression of pro-inflammatory *TNF-α* and *Vcam-1,* and protein expression of phospho-p65 subunit of the pro-inflammatory transcription factor NF-κB were particularly elevated in the more severe model of T2D (high-sucrose HFD with low-dose STZ), whilst pro-oxidant $p22^{phox}$ protein expression was elevated in both T2D rat models.
Discussion. Pro-oxidant/pro-inflammatory profiles were increased in T2D human ECs regardless of milieu *in vitro*. T2D rats on a high-sucrose HFD had an inflammatory and oxidant profile that more closely resembled those in T2D human ECs than the more moderate T2D model. These findings indicate that the more severe preclinical rat model of T2D (high-sucrose HFD with low-dose STZ) is a suitable model for cardiovascular complications of human T2D.

429. Innovative educational program to enhance pharmacy student knowledge and confidence in pharmacogenomics

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Introduction. The lack of uptake of pharmacogenomics (PGx) in clinical practice is associated with poor knowledge and confidence. Pharmacists play a key role in the implementation of PGx services. Innovative pedagogical approaches are required to develop the competence of pharmacy students, to steward PGx-guided medication management.
Aims. To evaluate the impact of an innovative educational program on pharmacy student knowledge, attitudes and confidence in PGx.
Methods. Second-year pharmacy students (n = 278) at the University of Sydney participated in a PGx module that consisted of lectures (5 hours) and a workshop (2 hours) with case-based learning. Student knowledge, attitudes and confidence in PGx were assessed before and after delivery of the education using the same survey. The survey consisted of 41 questions that were scored using a 6-point Likert scale. Results from surveys were compared using descriptive statistics. Focus groups were also conducted to gain further insights into survey responses.
Results. Pre- and post- survey response rates were similar (96% and 90%, respectively). Although 80% of students reported improvements in their PGx knowledge and skills, 83% requested more activities in the application of PGx information, using case-based learning. 84% of students felt "slightly, somewhat or extremely comfortable" in interpreting PGx test results. However, only 72% reported being confident in designing a PGx-guided dosing regimen. Communication of PGx-guided recommendations to other healthcare professionals as part of interprofessional collaboration improved by 41% to 78% . Qualitative analysis highlighted student interest in using role play simulations and personal PGx testing to support their learning. However, 58% of students expressed concerns about the ethical use of their own PGx data. Objective measures in knowledge will be assessed in final examinations.
Discussion. This study demonstrates that innovative pedagogy, specifically interactive case-based learning, can enhance PGx knowledge and confidence in applying new PGx knowledge in pharmacy students. Problem-based activities and self-PGx testing may further support student learning. Further exploration of the ethical implications associated with personal PGx testing is required.

430. Insights from student justifications for the choice of responses to multiple-choice questions

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Introduction. Drug interaction databases are designed to improve patient safety but do not address the reason(s) for which interactions occur. Enhancing students' ability to work from pharmacology first principles to understand these relationships could bolster confidence in clinical decision-making. Although multiple-choice questions (MCQs) can be designed to incorporate common misconceptions, they don't provide insight into the reason(s) for which a given response was chosen or the decision-making that underpins it. Incorporating an integrative approach comprising a brief justification of each answer requires students to address how they synthesised and applied their learning.
Aims. To design a series of MCQs to assess students' ability to integrate two mechanisms of action to explain drug interactions and to evaluate the justifications for insight into the students' decision-making process.
Methods. New MCQs were created, then checked for content and face validity by two pharmacy academics. Students were provided with exemplars, as well as an in-depth discussion of the approach, as a supplement to the weekly problem-solving activities. Inductive thematic analysis was used to evaluate the responses from two cohorts of students for insights into the thought processes and sources accessed to formulate the justifications.
Results. Students scored an average of 3 marks lower on the justifications than the MCQs. Most students struggled to articulate their rationale, with high-scoring students experiencing more difficulty. Almost one-third of students consistently searched for answers rather synthesising their learning and informal student feedback suggested that experience with more fact-based MCQ quizzes might have motivated this approach. A small cohort of students saw no reason to provide a justification when they had the correct answer, while others valued the opportunity to practice their critical thinking and analytical skills.
Discussion. Overall, students do not appear to have transferred the experience with the problem-solving activities embedded in the unit to answering and justifying the MCQs. Conversely, high scoring students might have been responding in a more intuitive manner, making it difficult to articulate their process. Responses highlighted certain misconceptions about the content, particularly the actions of different drug targets and how those targets affect cellular processes. In future, think-aloud interviews and focus groups will be conducted to provide additional insight into the reason(s) for which students struggled to justify their answers.

431. Can students identify mistakes when watching videos?

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Problem. We are now primarily teaching Generation Z students; they are digital natives who prefer to obtain information via online resources such as videos.(1) These videos may be from unreliable sources containing incorrect information, but can the students critically analyse information received and identify these mistakes? In academia, assessment tasks are still mainly using the traditional format where the focus is to assess if the students can analyse written information. In addition, this format is now largely challenged by the availability of natural language processing tools driven by artificial intelligence technology.
Solution. An online assessment task was designed, which supports delivery for large cohorts. Instead of written, multiple short videos presenting case studies with pharmacology mistakes were developed and embedded into quizzes. Students were expected to identify the mistakes and correct them. Simple and complex mistakes were used in the videos to assess students' different levels of knowledge. For example, utilising the incorrect word is considered a simple mistake, while the wrong interpretation of results is a complex mistake because it needs a deeper understanding and application of knowledge.
Impact and conclusion. This assessment task has now been successfully delivered online in three sessions. It was identified that this innovative format led to lower marks. Interestingly, the marks had a different distribution to previous quizzes, meaning some students performed better in this format while others performed considerably worse. The current artificial intelligence technology is unlikely to be helpful due to the way the information is presented and the quiz being time limited. In conclusion, it is crucial to develop assessments that test skills relevant to digital native students and simultaneously decrease academic integrity breaches. In addition, these results support the development of learning activities to help students practice those relevant skills.

(1) Szymkowiak, et al. (2021) Technology in Society.

432. Investigating the impacts of technology-enhanced learning strategies on the psychological motivation and performance of university students studying biomedical and health sciences

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Introduction. Many technologically-enhanced learning strategies have been retained in online and hybrid learning formats following the rapid uptake and reliance on technologies necessitated by COVID-19 restrictions to delivery in higher education. However, the literature is limited in providing insight into the impacts of these technologically-enhanced learning strategies on student motivation,engagement, and academic performance.
Aims. This study aimed to investigate the impact of technology-enhanced learning strategies on student self-reported intrinsic motivation, experience, engagement, and academic performance.
Methods. A questionnaire was undertaken to capture student self-reported intrinsic motivation[1] and academic performance[2], underpinned by theories of self-determination and self-efficacy. Demographic, Likert scale, select from list, yes/no, and open-ended question types were implemented. Students were recruited across a range of biomedical science and healthcare degrees at The University of Sydney (HREC approval 2023/432).
Results. Data are expressed as (mean +/- SD, median). Preliminary results show that participants (n=51) felt that Google Suite most enhanced their overall intrinsic motivation (4.6 +/- 0.6), as well as subscale factors of competency (4.6 +/- 0.6, 5) and autonomy (4.3 +/- 0.8, 5). On the other hand, social media forums and group chats had the greatest impact on relatedness (3.5 +/- 0.7, 5), and curiosity was best served using Anki, Kahoot, and other gamification tools (4.0 +/- 1.1, 5). Concerning academic performance, technological learning tools in general most improved learning strategies (3.3 +/- 1.2, 4), and least improved working in groups (2.8 +/- 1.4, 3).
Discussion. Current findings show that different learning technologies reinforce different aspects of intrinsic motivation and have different impacts on students' academic performance. These findings permit practical recommendations on the effective use of technologies under investigation.
[1]Ryan, RM (1982). Journal of Personality and Social Psychology, 43, 450-461.
[2]Greco, A et al. (2022). Frontiers in Psychology, 12, 498824-498824.

434. Implementation of a professional practice unit to prepare for industry-based work-integrated learning

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Introduction and Aim. The industry-based Pharmaceutical Science Honours degree at RMIT University allows students to put theoretical skills acquired as an undergraduate into practice with a 40-week work-integrated learning placement within the pharmaceutical industry. Placements are offered by pharmaceutical companies, contract research organisations, universities, and hospitals. Entry into the Honours program requires completion of a relevant undergraduate degree and successfully gaining a placement position by interview.
We aimed to create a structured program of career and professionalism skills, embedded in a core unit in the final semester of an undergraduate degree, to ensure that every student has the necessary tools to be prepared for professional practice at interview and within their industry-based placement.

Methods. Preparation of a professional CV and LinkedIn page along with guided analysis of position descriptions and organizations ensured students could present themselves and their knowledge of organizations and roles successfully at interview. Interactive workshops with peer-to-peer feedback exposed students to introductory, skills-based, and behavioural interview questions. A professional mock interview with academic staff and instant feedback put these skills into practice. Discussions and activities on ethics, patient/participant privacy, and conduct in the workplace provided students with a foundational base of knowledge around professional expectations.

Results and Discussion. Overall students were enthusiastic about preparing for their future careers and positively engaged in the course. Interview skills showed significant improvement after the peer-feedback collaborative learning workshops resulting in positive feedback from academics and industry. There was increased engagement and professionalism as students progressed through the course, demonstrating that a structured program of targeted career and professional development activities can enhance student preparedness for work.

435. The effect of compulsory indications in electronic hospital prescriptions on prescriber behaviour

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Introduction. Recording the indication for a medicine in the prescription supports communication and reduces errors. In electronic prescriptions the indication field can be made compulsory. However, compulsory fields risk inaccurate information being recorded. On 29/05/2023 our local health region made the indication field in prescriptions compulsory in the hospital prescribing system. This provided an opportunity to evaluate the effect of a compulsory indication field on prescriber behaviour. The text 'to be determined' was introduced in a drop-down selection box for use when the indication is unknown.
Aims. To evaluate making the indication field compulsory for all medicines in the hospital prescribing system.
Methods. The change in the proportion of prescriptions with an indication was compared for eight weeks after introduction of a compulsory indication field on 29/05/2023 to an equivalent eight-week period in 2022. Text in the indication field was manually classified as an indication, 'other text', 'rubbish text', 'to be determined', and blank. For prescriptions with 'to be determined' in the indication field, the proportion with an indication added, the dose changed, or the prescription ceased prior to discharge was measured.
Results. We analysed 81,646 prescriptions before and 83,427 after indications were made compulsory. The proportion of prescriptions with an indication increased from 29.2% to 78.1% ($p<0.01$). 'Other text' increased from 2.5% to 11.9% ($p<0.01$), 'rubbish text' from 0.0% to 2.7% ($p<0.01$) and 'to be determined' from 0.0% to 6.6% ($p<0.01$). Of 6,343 prescriptions with the indication 'to be determined' in the initial prescription, 5.6% were assigned an indication, 5.2% had the dose changed, and 20.8% were ceased, all prior to discharge.
Discussion. Introduction of compulsory indications for medicines increased recording indications in prescriptions substantially, with small increases in other text and rubbish text. Use of the 'to be determined' drop-down selection box rarely led to recording the indication prior to discharge and there was negligible effect on deprescribing.

436. Evaluating deprescribing recommendations at scale in discharge summaries using natural language processing

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Introduction: Deprescribing, a process of withdrawing inappropriate medication(s) supported by health care professionals with the goal of improving outcomes, is important for older adults with polypharmacy to avoid adverse drug events. Hospital admissions may provide an opportunity to assess the risk of medication-related harm and recommend deprescribing to General Practitioners (GPs) through discharge summaries. To assess and improve communication of recommendations from hospitals to GPs, efficient approaches to extract deprescribing recommendations from unstructured, free-text discharge summaries are needed.
Aims: To 1) develop a Natural Language Processing (NLP) model for extracting deprescribing recommendations from discharge summaries, and 2) evaluate how deprescribing recommendations for medicines with sedative and/or anticholinergic effects, as measured by the Drug Burden Index (DBI), were communicated in discharge summaries.
Methods: To develop the rule-based NLP model, discharge summaries were collected from 263 admissions of patients aged ≥75 years with DBI>0 at the time of review, admitted under general or geriatric medicine for >48 hours in an Australian tertialy hospital (13 July -31 October 2021). The NLP model included functions for recommendation extraction, misspelling detection, drug code matching, and DBI-contributing drug verification. Once the model performance reached a satisfactory level, the NLP model was applied to all 263 discharge summaries for the analysis.
Results: The model achieved a precision of 0.79, recall of 1.0, and F1 score of 0.88. Among the discharge summaries, 19% had deprescribing recommendations for DBI-contributing drug(s), with opioids being the class recommended most frequently. The DBI score on discharge was associated with the likelihood of deprescribing recommendations. Of 62 recommendations, 63% were documented in the discharge plan section with others in several different parts of the summary. The term "wean" was most frequently used for deprescribing.
Discussion: Our NLP model efficiently extracts deprescribing recommendations from discharge summaries. Deprescribing recommendations are frequent but lack uniformity and specificity in communication. Future studies could evaluate the model's generalisability across different hospitals and services.

437. Evaluation of Partnered Pharmacist Medication Charting at SALHN

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Introduction. Partnered Pharmacist Medication Charting (PPMC), a collaborative pharmacist-doctor approach to create a shared medication treatment plan on admission and subsequent pharmacist-led charting of medications, has been shown to reduce medication charting errors, medication-related harm, and length of stay (LOS) in other Australian states.
Aims. To evaluate the impact of the PPMC project on LOS, medication charting errors and harm associated since implementation at a tertiary hospital.
Methods. We conducted a quasi-experimental study assessing the effect of PPMC on medication charting and clinical outcomes. Patients admitted to the Acute Medical Unit or General Medicine at Flinders Medical Centre (SALHN) were divided into PPMC vs. standard care. Selection to PPMC was non-randomised, non-blinded and largely dependent on medication regimen complexity determined by the PPMC pharmacist. We assessed LOS, hospital-acquired complications (HACs), venous thromboembolism (VTE) prophylaxis charting rates, and medication charting errors in both groups.
Results. Since PPMC introduction in 22nd March 2022, there have been 216 patients had PPMC and 7,179 standard care. The average LOS was 5.25 days for control vs. 4.27 days (P = 0.144) for PPMC. Subgroup analysis of average LOS for patients with >5 pre-admission medications (n=3,988 for control; n=200 for PPMC) and the same with age >65 years (n=3,022 for control; n=152 for PPMC) was 6.38 vs. 4.38 (P=0.005) and 6.50 vs. 4.54 days (P=0.002) respectively. A total of 17 medication-related HACs (HAC 10) were recorded in the control cohort vs. none in the PPMC cohort (P=1). Overall, VTE prophylaxis charting rates (including those on an anticoagulant pre-existing) were 52% in control vs. 75.6% (P=0.002)in PPMC. The chart error rate was 85% in control vs. 15% in PPMC, with an average of 27.8 errors occurring for every 100 orders with standard prescribing compared to 1.3 through PPMC (P < 0.001).
Discussion. PPMC in SALHN was associated with significant improvements in medication charting and reduced LOS comparable to interstate experience. Further prospective evaluation is planned in 2023.

438. People with dementia and carers' medication management information needs: A scoping review

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Introduction. People with dementia experience harmful effects of medications more than people without dementia and, along with their carers, have indicated they need better guidance on medication management.
Aims. To review literature identifying the priorities for medication management information expressed by people with dementia and carers.
Methods. A search was conducted from inception to 12 May 2023 in Medline, Embase, Cochrane Library, PsychINFO, Web of Science, CINAHL, and Ageline for studies that reported the information needs of people with dementia and/or carers regarding medication management. Data were extracted by the seven aspects of medication management: select, supply, prepare, administer, record, monitor, and review medications. Study characteristics were summarised quantitatively, and themes were extracted using content analysis to explore the information priorities for medication management guidance. Two reviewers independently screened the abstracts and full-texts, and extracted data.
Results. Of the 11367 records screened, 36 studies were included. Six preliminary information priorities have emerged: administration guidance (for when there are swallowing difficulties or medication refusal), knowing what the effects of medications look like (including side effects), the indication and duration of medications, optimising continuity of care between healthcare settings (keeping documents up-to-date), balancing the potential benefits and risks of medications (especially high-risk medications), and acknowledging self-determination of the person with dementia so they can be involved in medication discussions. The most commonly reported information needs were monitoring, administering, and selecting medications.
Discussion. Our preliminary findings suggest that people with dementia and carers need more tailored guidance on medication management that clearly describes the benefits and harms of medications so they can make informed choices and participate effectively in shared decision-making.

439. Dosing errors with paracetamol in Australians aged 12 and over, 2017-2023

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Introduction. Paracetamol is a widely used medication worldwide and frequently used in overdoses due to its accessibility. The NSW Poisons Information Centre (NSWPIC) answers and provides advice for over half of all national calls to the Poisons Information Hotline, where those attributed to paracetamol are becoming more frequent.
Aims. To describe patterns and characteristics of dosing errors with paracetamol reported to the NSWPIC in individuals aged 12 and above including demographics, types of errors, products involved, reasons for ingestion, hospitalisation, the need for n-acetylcysteine, hepatotoxicity, liver unit referral and death.
Methods. Calls involving paracetamol coded as a therapeutic error or intentional exposure (non-deliberate self-poisonings) between January 2017-June 2023 were extracted from the NSWPIC database. Data underwent general cleaning before a closer look at 2021 data which was extracted into a preformatted spreadsheet.
Results. NSWPIC provided advice for 24644 individual exposures for dosing errors attributed to paracetamol between January 2017 and June 2023. 14380 (58.4%) of these exposure calls were for those aged >12. The most common forms of paracetamol involved in both the entire cohort and 2021 alone included immediate release (51.8% vs 60.2%) and modified release (25.6% vs 30.5%) paracetamol. Multiple products were taken in approximately 20% of both cohorts. In 2021 73.0% of people were able to be managed at home/in the community and those who needed to be hospitalised had a greater median dose of paracetamol/24h period (8.0g vs 3.0g).
Discussion. Accidental paracetamol poisoning is still a large and growing problem for Australians with many still requiring hospitalisation and treatment. The number of formulations and combinations adds to increasing confusion surrounding appropriate dosing. Additionally, the magnitude of dosing errors may also reflect inadequate pain relief however, with a narrow safety margin there is little room for error.

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440. Efficacy and Safety of Opioids for Osteoarthritis: Systematic Review and Meta-Analysis.

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Introduction. Opioid analgesics are commonly prescribed for osteoarthritis. Guidelines provide inconsistent recommendations on the use of opioid analgesics in osteoarthritis and previous reviews are limited in scope, warranting a comprehensive assessment of the evidence in this area.

Aims. To evaluate the efficacy, safety and dose dependent effects of opioids for osteoarthritis compared with placebo.

Methods. This was a systematic review and meta-analysis. Electronic databases were searched (inception to October 2020) for randomised placebo-controlled trials evaluating any opioid analgesic for osteoarthritis. The primary outcome was pain at the medium term (≥6 weeks but <12 months). Continuous pain and disability outcomes were converted to a 0 to 100 scale. Effects <10 points were considered very small, 10-19 points small, 20-29 points moderate and >30 points large. Dichotomous outcomes were presented as risk ratios (and 95% confidence intervals, CI). Four authors extracted data and assessed bias. Data were pooled using a random effects model. Quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE).

Results. Thirty-six trials (dose range: 10-210 oral morphine milligram equivalent units/day) were included. For the *medium term*, there was low quality evidence from 19 trials (n=8965 patients) of a very small effect of opioids compared to placebo for pain; mean difference (MD) -4.59 (95% CI -7.17, -2.02) and low quality evidence from 16 trials (n=6882) of a very small effect on disability; MD -4.15 (95% CI -6.94, -1.35). Meta-regression did not show a significant association of opioid dose with adverse events or pain relief. Opioids increased the risk of adverse events; RR: 1.43 (1.29, 1.59), but evidence was of very low quality. There were no long-term outcomes data.

Discussion. For people with osteoarthritis, opioids may provide very small effects on pain and disability, and may increase the risk of adverse events. The relationship between opioid dose, pain relief and risk of adverse events requires further evaluation.