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Oral Abstracts

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Wither mechanism-based modeling of drug action?

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The objectives of this presentation are to discuss fundamental principles of mechanism-based modeling, highlight examples of useful applications, and to describe the role of such modeling in drug discovery, development, and therapeutics. A major goal of clinical pharmacology is to identify sources of variability in individual therapeutic and adverse responses to drugs. There have been considerable advances in accounting for the variability in drug exposure (pharmacokinetics or PK); however, far fewer examples exist in which patient characteristics (or covariates) have been linked to pharmacodynamic (PD) variability. Sources of PD variability include (but are not limited to): target-binding interactions (affinity and expression/turnover), presence of endogenous ligands, signal transduction, adaptive feedback (or homeostasis), and disease progression. Traditional PK/PD models often utilize compartmental structures to integrate the time-course of drug exposure, pharmacological properties (capacity, sensitivity, and transduction of drug-target interactions), and (patho-) physiological turnover processes. These models can be coupled with nonlinear mixed effects modeling (i.e., *pharmacometrics*) to identify patient specific covariates (e.g., genetic polymorphisms) that explain the inter-individual variability in exposure-response relationships. However, empirical and semi-mechanistic PD models are rarely sufficiently robust (regardless of model performance) to prospectively predict combinatorial drug responses, complex genotype-phenotype relationships, off target toxicity, and approaches to multidimensional datasets. In contrast, *systems pharmacology* is an emerging field that integrates systems biology and PD modeling to predict and resolve the complex interactions between drugs and biological systems that underlie emergent properties of cells, tissues, and whole organisms. Both drugs and disease processes give rise to complex and dynamic clinical phenotypes by altering natural interconnected biochemical networks and support the emergence of systems pharmacology models of drug action. Strategies are needed to efficiently utilize both pharmacometrics and systems pharmacology in realizing precision and individualized therapy.

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Molecular and structural basis of the allosteric control of GPCR phenotype by receptor activity-modifying proteins (RAMPs)

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The calcitonin family of peptides includes calcitonin (CT), calcitonin gene-related peptides (CGRPs), amylin and adrenomedullin (AM) peptides. These peptides play important physiological roles, including regulation of bone and glucose metabolism, cardiovascular development and response, and reproduction. The receptors for these peptides arise from just two G protein-coupled receptors from the class B subfamily, the CT receptor (CTR) and CT receptor-like receptor (CLR), with numerous distinct receptor phenotypes encoded through heterodimerisation of each of these GPCRs with one of 3 receptor activity-modifying proteins (RAMPs 1-3). I will discuss historical data on distribution of these receptors that led to the discovery of novel receptor phenotypes that were later ascribed to the RAMP:CTR and RAMP:CLR complexes. More recent data on the molecular control of efficacy and the structural basis for formation of the CT family of receptors will also be discussed.

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Both β_1- and β_2-adrenoceptors mediate arrhythmic contractions, ryanodine receptor channel opening and phosphorylation in human failing hearts:- control by PDE3

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Introduction. Heart failure (HF) is a global health issue with rising prevalence, affecting around 26 million people worldwide. In Australia, the prevalence of HF is 1.0-2.0% of the population, increasing to over 10% in patients 75 years or older. Despite advances in medications, mortality and readmission rates have remained largely unchanged with survival rates of ≤ 40% at 5 years from diagnosis. Sudden cardiac death (SCD) from ventricular arrhythmias (VA) is the leading mode of death (45-50%). Activation of the sympathetic nervous system contributes to the genesis of dangerous ventricular arrhythmias.

Aims. 1. To determine the contribution of b_1- and b_2-adrenoceptors to arrhythmic contractions in a model of VA in explanted human ventricle from patients with heart failure. 2. To determine the control of VA by phosphodiestereases (PDE). 3. To determine the contribution of ryanodine receptor (RyR2) channels to VA.

Methods. Human right ventricular trabeculae were clamped to an electrode block, the other end attached to a strain gauge transducer and electrically stimulated to contract. The stimulator was turned off to observe arrhythmic contractions caused by (-)-noradrenaline or (-)-adrenaline through activation of b_1- or b_2-adrenoceptors respectively in the absence or presence of the PDE3 inhibitor cilostamide. Trabeculae were snap frozen and RyR2 channels reconstituted in lipid bilayers to determine channel opening. RyR2 phosphorylation was determined by Western Blot.

Results. Activation of both b_1- and b_2-adrenoceptors caused arrhythmic contractions ($P < 0.01$) which were potentiated by cilostamide ($P < 0.01$). Both b_1- and b_2-adrenoceptors increased diastolic RyR2 channel opening and phosphorylation, which correlated with altered levels of RyR2 phosphorylation at Ser-2808 and Ser-2814.

Discussion. Activation of both β_1- and β_2-adrenoceptors mediates arrhythmic contractions, RyR2 channel opening and phosphorylation in ventricle of human failing hearts. Arrhythmias were controlled by PDE3.

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Characterising prolonged high fat diet as a model of obesity-induced left ventricular diastolic dysfunction in mice.

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Introduction. The prevalence of heart failure (HF) with preserved ejection fraction (HFpEF) is increasing, particularly in the aged and diabetic population. The causes of cardiac dysfunction are multifactorial and likely reflect increased diabetic cardiomyopathy, characterised by left ventricular (LV) diastolic dysfunction and later systolic dysfunction, both not detected until later stages of disease progression.

Aims. To gain insights into contributing factors of altered cardiac function in an age-appropriate model of HFpEF.

Methods. Male FVB/N mice commenced a high fat diet (HFD) from either 6 or 26 weeks of age and all mice received streptozotocin (55mg/kg/d) or citrate vehicle i.p. for 3 days at 26 weeks of age. At 64 weeks of age (study endpoint), LV function via Doppler echocardiography and pressure-volume relationship (PVR) were determined under anaesthesia (ketamine/xylazine/atropine: 80/8/0.96mg/kg i.p.), followed by tissue collection. Data are mean ± SEM. *P<0.05: citrate vs STZ within diet; #P<0.05: citrate chow vs HFD (6 or 26 wks); ^P<0.05: STZ chow vs HFD (6 or 26 wks)

Endpoint characteristics (64 weeks of age)	Chow diet (from 6 wks of age)		HFD (from 6 wks of age)		HFD (from 26 wks of age)	
	Citrate	STZ	Citrate	STZ	Citrate	STZ
Body weight (g)	39.1±1.1 (10)	35.7±0.9 (7)	54.1±2.4 (11) #	38.8±3.9 (4) *	48.9±1.2 (15) #	40.6±1.3 (5) *
Blood glucose (mmol/L)	9.0±0.6 (10)	16.6±3.3 (7) *	7.8±0.4 (11)	9.8±2.8 (4) #	9.4±0.5 (15)	14.7±3.2 (5) *
LV weight/tibia length (g/mm)	6.4±0.1 (10)	6.2±0.1 (7)	8.0±0.3 (11) #	6.4±0.5 (4) *	8.1±0.2 (15) #	6.2±0.3 (5) *
LV superoxide (RLU/mg) (fold)	1.0±0.2 (10)	2.7±0.5 (7) *	2.0±0.2 (9) #	2.6±0.4 (4)	2.3±0.3 (15) #	2.7±0.7 (5)
E/A ratio	2.53±0.24 (9)	2.29±0.23 (7)	1.34±0.07 (10) #	1.26±0.16 (4) ^	1.69±0.14 (16) #	1.45±0.13 (5) ^
A wave velocity (cm/s)	35.8±3.9 (9)	45.0±7.0 (7)	58.2±2.4 (10) #	57.0±6.3 (4)	53.4±4.1 (16) #	60.2±4.9 (5)
Ejection fraction (%)	59.7±4.1 (8)	59.9±1.9 (6)	60.3±3.2 (7)	57.0±4.3 (4)	60.1±3.3 (10)	71.0±5.3 (3)
End systolic pressure (mmHg)	123.0±6.3 (8)	102.9±5.8 (6)	101.4±11.8 (7) #	68.3±4.3 (4) *	99.8±5.9 (10) #	108.6±12.2 (3)
End systolic PVR (mmHg/µl)	4.7±0.8 (8)	3.2±0.6 (6)	2.1±0.3 (7) #	2.5±0.3 (4)	3.3±0.5 (10) #	3.4±0.6 (3)

Discussion. Long-term obesity commencing early in life (6 wks of age in mice) followed by later hyperglycaemia results in poor outcomes for cardiac function in terms of diastolic dysfunction (impaired E/A ratio) and reduced end-systolic pressures. Ejection fraction however was preserved, similar to HFpEF in humans. Whether these functional characteristics are accompanied by morphological characteristics of HFpEF, remains to be determined.

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Mechanisms underlying cognitive impairment during small vessel disease

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Introduction. Dementia is a major health problem in Australia and with the aging population, the burden of dementia is expected to rise. Small vessel disease (SVD) is associated with vascular dysfunction in pial and parenchymal arterioles, inflammation and a decline on cognitive function due to disruption of cerebral blood flow. Of the known risk factors for SVD, hypertension is the most prevalent.

Aims. To assess cognitive, microvascular and inflammatory changes in the brain during hypertension.

Methods. Hypertension was induced by either chronic infusion of angiotensin II (0.7 mg/kg/day) via osmotic minipump or deoxycorticosterone + 0.9% salt in the drinking water (DOCA-salt). Vascular function was assessed by pressure myography or *in vivo* using a cranial window. Brain inflammation was measured by flow cytometry. Working memory was evaluated using the novel object recognition test. Cerebral blood flow was assessed by laser speckle contrast imaging.

Results. Hypertension promoted accumulation of leukocytes in the brain, including neutrophils, monocytes, T cells and B cells, all of which were elevated by ~2.5-fold compared to vehicle-treated mice ($P<0.05$). Co-administration of hydralazine prevented hypertension ($P<0.05$) and blunted brain inflammation. Hypertension impaired endothelium-dependent dilation of both isolated parenchymal (baseline diameter of 15±1 µm) and pial arterioles (37±1 µm). Inhibition of angiotensin II type 1 (AT1R) or mineralocorticoid receptors (MR) or Rho kinase ameliorated endothelial dysfunction. Inner diameter of maximally dilated parenchymal arterioles was reduced approximately 20% by DOCA-salt treatment ($P<0.05$ vs sham). Vascular dysfunction and inflammation was associated with impaired working memory and disrupted cerebral blood flow in hypertensive mice.

Discussion. Hypertension promotes brain inflammation, microvascular dysfunction and cognitive impairment. Chronic brain inflammation and microvascular dysfunction are key pathological mechanisms in the pathogenesis of SVD and would represent therapeutic targets.

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Impact of diabetes on the murine cardiac cellular landscape and systemic leukocyte proportions

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Introduction. Diabetes is the 9th leading cause of death worldwide. Approximately 90% of its incidence is attributed to type-2 diabetes (T2D), a highly complex, pro-inflammatory metabolic disorder. Diabetes is an independent cardiac risk factor, with >60% of T2D patients developing heart failure. Recent paradigm shifts in our knowledge of cardiac cellular composition have revealed the need for more detailed investigations in cardiovascular pathology.

Aims. Our objectives from this study were: (1) elucidate the shifts in the cardiac cellular landscape in murine T2D; (2) determine if circulating or tissue-specific leukocytes are associated with changes in cardiac cell populations.

Methods. T2D was induced in 6-week-old male FVB/N mice by low-dose streptozotocin (3x55mg/kg, i.p) and high-fat diet (HFD) for 26 weeks. Diastolic function was measured by Doppler echocardiography in anaesthetised mice (Ketamine/Xylazine/Atropine, 80/8/0.96mg/kg, i.p.). High-dimensional flow-cytometry was performed on cardiac ventricles as well as blood, spleen, liver, and bone-marrow from non-diabetic (ND, n=7) and T2D (n=19) mice.

Results. T2D mice exhibited elevated glycated haemoglobin and impaired glucose tolerance at endpoint. Diastolic dysfunction was evident, detected by E/A and e'/a' ratio. Endothelial cell (ECs; $CD31^+$) and smooth-muscle cell ($Mcam^+CD39^+$) proportions were significantly reduced in T2D mouse hearts. Conversely, resident mesenchymal cells (RMCs; $CD31^-CD45^-$) were significantly elevated as a result of T2D, likely due to the marked increase in cardiac fibroblasts (Mcam-). While circulating lymphocytes were unchanged, circulating myeloid cells were elevated, as well as reticulated (TO^+CD41^+) and mature platelets ($CD41^+$). Splenic $Ly6C^{hi}$ and $Ly6C^{lo}$ monocytes were also increased.

Discussion. Our data suggests a clear shift in the cardiac cellular ecosystem favouring increasing proportions of RMCs, possibly at the cost of EC loss, which could be induced by systemic leukocyte changes. We postulate that although resident cardiac leukocyte proportions are unchanged in this model of T2D, their autocrine and paracrine communications may be an important factor driving the cardiac cellular shifts which likely contribute to cardiac dysfunction in the context of T2D.

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Effects of long-term polypharmacy and deprescribing on cardiovascular function and cardiac structure in aged mice

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Introduction. Polypharmacy (concurrent use of ≥ 5 medications) and exposure to drugs with increasing Drug Burden Index (DBI: the cumulative exposure to anticholinergic and sedative drugs) are associated with impaired function in older adults. Preclinical studies can provide a mechanistic understanding of these exposures on organ function.

Aims. We aim to evaluate the effect of chronic polypharmacy, monotherapy with increasing DBI and deprescribing (cessation of medications) on cardiovascular function and cardiac histology in aged mice.

Methods. 12-month-old male mice received control or medicated feed containing polypharmacy regimens of Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol and irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol and citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone and citalopram) or monotherapy, with each medication independently from the High DBI regimen, all at therapeutic doses. At 21 months, animals were stratified to continue treatment or underwent deprescribing. BP was assessed every three months. Hearts were collected at age 26 months for histological studies.

Results. Animals tolerated the dose well. At 21 months, compared to control, systolic and diastolic BP decreased in Zero DBI, Low DBI, metoprolol and simvastatin treated mice ($P<0.05$) but not in High DBI (also contains metoprolol and simvastatin) group ($P>0.1$). Collagen quantification indicated that there was no significant difference detected in collagen content among all groups. Compared to control, left ventricular (LV) wall thickness was not significantly different following treatment. Consistently, compared to control, LV myocyte size (n=3 for control, High DBI and High DBI deprescribed; 50 cells were randomly measured in each sample) was also no different with treatment groups.

Discussion. Our results suggest that chronic treatment with this High DBI polypharmacy regimen may impair the therapeutic effects of antihypertensives. Future studies will continue to investigate morphological changes of the heart including cell size and damage.

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Cardiomyocyte ErbB4 receptor deletion causes rapid-onset cardiac failure in neonatal mice

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Background/Introduction: Activation of ErbB4 by neuregulin 1 (NRG1) promotes cardiomyocyte hypertrophy and proliferation in both adult and neonatal mice, while treatment of patients with NRG1 following myocardial infarction reduces scar size and improves function. In mice, deletion of ErbB4 from cardiomyocytes mid-gestation results in development of dilated cardiomyopathy and reduced survival, pointing to a critical role for ErbB4 in the heart. Purpose: We sought to determine critical period(s) for cardiomyocyte ErbB4 in the lifespan. Methods and Results: We deleted ErbB4 in αMHC-MerCreMer (cCre Tg+/)/ErbB4 homozygote floxed ($ErbB4^{fl/fl}$) mice at ~2 months of age with 10 injections of Tamoxifen (20 mg/kg/day). Contractile function was reduced *in vivo* (echocardiography, 16%) and *ex vivo* (isolated-perfused, 33%) 3 months after gene deletion, while survival in mice up to 8 months after tamoxifen treatment was not modified by cardiomyocyte ErbB4 deletion, and hearts retained robust responses to both physiological (exercise) and pathological (Angiotensin II) hypertrophic stressors. Taken together, this indicated that ErbB4 is not essential for survival and adaptation in the adult heart, pointing instead towards a critical window for ErbB4 in neonatal heart development. To test this hypothesis, $ErbB4^{fl/fl}$ and $ErbB4^{wt/wt}$ neonates were injected at P1 with AAV9-cTNT-eGFP-iCre (2.16x1011viral particles, temporal vein) and culled at P6. We confirmed the presence of iCre and eGFP mRNA in all AAV-injected mice, and suppression of ErbB4 in AAV-injected $ErbB4^{fl/fl}$ mice, but no changes in heart size, body weight or expression of the ErbB4 ligand neuregulin. On P8/9 AAV-infected $ErbB4^{fl/fl}$ mice exhibited a rapid-onset dilated cardiomyopathy associated with increased mortality, a doubling of heart size, decreased cardiomyocyte proliferation and compensatory upregulation of neuregulin expression. Conclusion: ErbB4 is critical to maturational cardiac hypertrophy in neonatal mice, and maintains adult heart function.

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Efficacy and safety of tapentadol immediate release for acute pain: a systematic review and meta-analysis

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Introduction: Opioids remain an important component of therapy for acute pain. Tapentadol immediate release (IR) is a newer opioid. However, evidence pertaining to its safety and efficacy compared to other opioids and its place in therapy are not well defined.

Aim: To conduct a systematic review and meta-analysis to examine the efficacy and safety of tapentadol IR compared to other short-acting orally administered opioids for the management of acute pain.

Methods: A systematic literature review was conducted using the Cochrane Library, Embase, International Pharmaceutical Abstracts, Medline, PubMed and Web of Science from inception to 17th May 2019 to include studies comparing the safety and efficacy of tapentadol IR use with other IR orally administered opioids.

Result: In total, 13 studies and one abstract were included in the systematic review (n=12,814 patients). Of these, eight RCTs (n=3,706 patients) comparing 50-100mg tapentadol IR versus 5-15mg oxycodone IR were included in the meta-analysis. The lowest dose of tapentadol IR (i.e. 50mg) was associated with less pain control compared to oxycodone IR (standardized mean difference 0.25, 95% CI 0.06 to 0.44). However, there were no statistically significant differences at higher doses (i.e. 75mg, 100mg or when a titration strategy was used). Pain control with tapentadol IR was also similar to morphine IR and tramadol IR. The dose of 50mg tapentadol IR was less likely to have adverse effects (ADEs) such as nausea (RR 0.60, 95% CI 0.48 to 0.75) vomiting (RR 0.39, 95% CI 0.29 to 0.53), constipation (RR 0.44, 95% CI 0.32 to 0.61) and dizziness (RR 0.62, 95% CI 0.51 to 0.76) compared to oxycodone IR. The doses of 75mg and 100mg tapentadol IR were superior to oxycodone IR with regard to nausea (75mg: RR 0.61, 95% CI 0.45 to 0.81; 100mg: RR 0.82, 95% CI 0.70 to 0.97) and constipation (75mg: RR 0.31, 95% CI 0.21 to 0.45; 100mg: 0.62, 95% CI 0.39 to 0.97). Tapentadol IR in titrated dose was associated with less constipation (RR 0.46, 95% CI 0.29 to 0.73).

Discussion: Tapentadol IR is as effective as other opioids at higher doses for acute pain and is associated with fewer gastrointestinal adverse effects. Based on these findings, tapentadol IR can be considered as a first line opioid for acute pain.

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Prevalence of adverse drug events and adverse drug reactions in hospital among older patients with dementia: a systematic review

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Introduction. Older people with dementia are high users of acute care services. There is a high prevalence of adverse drug events (ADEs) and adverse drug reactions (ADRs) among older inpatients with dementia, potentially leading to negative health outcomes including further cognitive decline, delirium and falls.

Aims. This systematic review aimed to quantify the prevalence of ADEs and ADRs in older inpatients with dementia.

Methods. A systematic search of observational studies was performed in Embase, Medline, PsycINFO, International Pharmaceutical Abstracts, Scopus and Informit from inception to May 2019. Articles published in English that reported the prevalence of ADEs or ADRs in hospital patients aged 65 years or older with dementia were included. Two authors reviewed titles and abstracts and all eligible full-text articles. Relevant information relating to ADEs, ADRs and dementia were obtained from each article.

Results. A total of five articles were included. Only one study reported the prevalence of ADEs to be 81.5%, defined using the Naranjo algorithm. Four studies assessed the prevalence of ADRs, ranging from 12.7% to 24.0%, assessed using various methods. One study defined ADRs according to the World Health Organization-Uppsala Monitoring Centre (WHO-UMC) criteria, two studies employed the WHO definition and one study did not explicitly define ADRs. The most frequently reported drug classes implicated in ADRs were psychotropic, antihypertensive and analgesic drugs, implicated in up to 60.0%, 20.0% and 18.0% of ADRs respectively.

Discussion. Our findings suggest that ADEs and ADRs are common in older inpatients with dementia. However, only one study documented ADEs and there was variability in approaches to ADR assessment. A greater understanding of ADEs and ADRs, as well as tailored assessment tools, will promote prevention of ADEs and ADRs in people with dementia.

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Missing data reporting in clinical pharmacy research

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Introduction. Missing data is common in clinical research and presents as a pervasive challenge for researchers. The scientific accuracy of studies, reliability of outcomes, and applicability to the real-world is threatened by missing data.

Aims. Our study aimed to document the ways by which missing data were handled in clinical pharmacy research to provide an insight into the amount of attention paid to the importance of missing data in this field of research.

Methods. Ten journals affiliated with pharmacy organizations in the United States, Canada, United Kingdom and Australia were evaluated. Randomized controlled trials, cohort studies, case-control studies and cross-sectional studies published in 2018 were included. The primary outcome measure was the proportion of studies that reported the handling of missing data in their methods or results.

Results. A total of 178 studies were included in the analysis. Of these, 19.7% (n=35) mentioned missing data either in their methods (3.4%, n=6), results (15.2%, n=27) or in both sections (1.1%, n=2). Only 4.5% (n=8) of studies mentioned how they handled missing data, the most common method being multiple imputation (n=3), followed by indicator (n=2), complete case analysis (n=2) and simple imputation (n=1). One study using multiple imputation and both studies using indicator method also combined other strategies to account for missing data. One study only used complete case analysis for sub-group analysis and the other study only used this method if a specific baseline variable was missing.

Discussion. Very few studies in clinical pharmacy literature report any handling of missing data. This has the potential to lead to biased results. We advocate that researchers should report missing data and how it was handled to increase the transparency of findings and minimize potential bias. Furthermore, the editorial process of journals should passively encourage adherence to reporting guidelines and incorporate methods to detect compliance.

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Pharmacological Treatment Dynamics in People Initially Prescribed Metformin or Sulfonylurea for Type 2 Diabetes

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Aim. To estimate the predictors of time to anti-glycaemic medication addition and switching during the first year after initiation of metformin or sulfonylurea (SU) in people with Type 2 Diabetes (T2DM).

Method. 109,573 individuals aged 18-99 years initiating metformin or a SU between July 2013 and April 2015 were identified from the National Diabetes Service Scheme (NDSS) database containing 80-90% of Australians with diagnosed T2DM. Medication use was accessed via pharmacy dispensing data linked to the NDSS. Cox Proportional Hazards Regression was used to estimate adjusted Hazard Ratios (HRs) with 95% confidence intervals (CI) for predictors of time to an addition to or switch from the index medication during a one-year follow-up.

Results. Addition or switching occurred in 18% and 4% of metformin initiators and in 28% and 13% of sulfonylurea initiators, respectively. People aged ≥75 years versus 18-49 years (HR 0.56; 95%CI 0.53—0.60) had a lower risk, while those with Congestive Heart Failure (CHF) (HR 1.25; 95%CI 1.13—1.39) had a higher risk of an addition to metformin. Switching from metformin occurred faster in people with ≥5 comorbidities versus none (HR 1.52; 95%CI 1.30—1.77) but slower in Australia's most remote locations versus major cities (HR 0.73; 95%CI 0.56—0.95). Time to addition to sulfonylureas was longer in people aged ≥75 years versus 18-49 (HR 0.44; 95%CI 0.37—0.51), with ≥5 comorbidities versus none (HR 0.59; 95%CI 0.48—0.73) and living in the most remote areas versus major cities (HR 0.69; 95%CI 0.52—0.92). Longer durations up to 2 years from diagnosis to the initiation of metformin or sulfonylurea were associated with longer time to receiving an addition to or switch.

Conclusion. Longer intervals (≤2 years) before metformin or sulfonylurea initiations after diagnosis reduce the likelihood of individuals receiving an addition to or switch from the initial medication within one year. People in Australia's most remote areas are less likely to receive a switch from metformin or an addition to sulfonylurea than those in major cities.

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Pharmacovigilance Study of clozapine in New Zealand using three outcomes databases

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Introduction. Clozapine is currently used in New Zealand for treatment-resistant psychosis and there are proposals to extend its usage. However, there are also also well-documented risks including serious metabolic, cardiac, haematological, and gastrointestinal adverse reactions (ARs) associated with clozapine.

Aims. The present study aimed to describe the incidence of serious ARs with clozapine use in New Zealand.

Methods. Exposure to antipsychotics was identified from the New Zealand National Pharmaceutical Collection. Outcomes were identified from three sources: the Suspected Medicine Adverse Reaction Search (SMARS) database of Medsafe, the National Coronial Information System (NCIS) and Hospital Separation Data. Data were linked using encrypted national health numbers. Data were analysed using descriptive statistics and incidence rates (95% CI). All data were de-identified, and the research was given ethics approval at an institutional level.

Results. For the time period 2007 to 2015, there were 5,755 patients who used clozapine compared with 4,528,875 non-users of antipsychotics included in the analysis. Clozapine users represented 2.5% of the 233,068 patients treated with antipsychotics over this time period. The incidence rate ratio (95% CI) for clozapine compared with non-users of antipsychotics (adjusted for age, gender and comorbidity) was 4.04 (3.04-5.36) for arrhythmia, 2.75 (1.59-4.75) for myocarditis, 0.33 (0.11-1.03) for ischaemic heart disease, 1.97 (0.27-14.18) for hepatic outcomes, 7.55 (6.04-9.44) for gastrointestinal outcomes, 3.34 (2.53-4.43) for metabolic outcomes, 3.22 (1.44-7.24) for dermatological outcomes, 6.43 (3.53-11.73) for haemopoietic outcomes, and 1.75 (1.08-2.83) for renal outcomes. In the SMARS database, from 2007 to 2015 there were 5,141 reports related to antipsychotics with 1,877 (36.5%) related to clozapine. Clozapine was related to 132 (67.3%) reports of neutropenia, 72 (70.6%) of myocarditis and 53 (74.6%) of constipation. In the NCI database, from 2008 to 2012 there were 31 deaths associated with clozapine, 24 with olanzapine, 21 with quetiapine and <5 with risperidone.

Discussion. Clozapine use is associated with considerable morbidity and mortality which may limit its use to severe/resistant psychosis.

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Recommendations of pharmacological alternatives for anticholinergic class of medications prescribed to individuals with dementia

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Introduction. Anticholinergics have been notorious for their association with worsening of cognitive impairment in individuals diagnosed with dementia. Utilizing the international Resident Assessment Instrument-Home Care (interRAI-HC) tool, we found that a fairly high number (67%) of potentially inappropriate medications were prescribed in the subset of older adults with dementia, most of which belonged to the anticholinergic class of medications (60%).

Aim. To assess medication appropriateness in older adults with dementia and develop a guideline for the alternatives to currently prescribed potentially inappropriate anticholinergic class of medications.

Methods. A literature review of the anticholinergic burden scales and serum anticholinergic activity of various medications was undertaken, and we formulated a guideline for prescribers, emphasizing the need for pharmacological alternatives to medications with anticholinergic properties for individuals with dementia presenting with co-morbidities.

Results. Of the 117 medications prescribed for ailments of the Central Nervous System, 38% were classified as medications with high or moderate anticholinergic activity (HOMAA), and 56% were observed to possess low or no anticholinergic activity (LONAA). Likewise, for the gastrointestinal, cardiovascular, respiratory, endocrine, genito-urinary system, and infections; we found that of all medications prescribed, those which were observed to have HOMAA constituted 28%, 3%, 46%, 0, 5%, 30% respectively, and the medications which possessed LONAA comprised 48%, 56%, 43%, 62%, 43%, 70% respectively.

Discussion. The implementation of this guideline for prescribing alternatives to anticholinergic medications in this vulnerable population has the potential to reduce adverse effects linked with the prescription of anticholinergic medications, slower cognitive decline, and thereby reduce the risk of mortality. As a step further in this ongoing research, we are utilizing the interRAI assessments for validating the guideline in older adults with dementia.

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A population pharmacokinetic model of mycophenolic acid using dual-phase absorption and plasma protein binding

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Introduction. Bayesian dosing of mycophenolate mofetil has shown clinical benefit using a dual-phase absorption pharmacokinetic (PK) model. Elsewhere, mycophenolic acid (MPA) population PK models have described changes in plasma protein binding using a non-saturable K_B parameterisation. This is critical in the initial post-transplant phase, where dynamic changes in renal function and serum albumin can alter the relationship between total MPA and the 'effective' unbound MPA, as confirmed in vitro.

Aims. To develop a population PK model in an Australian kidney transplant population, using total and unbound MPA concentrations and a dual absorption structure.

Methods. The ADOPT Trial (Dose Optimization Prior to Transplant) collected unbound and total MPA data from 45 transplant recipients, peri-transplant and in the initial post-transplant weeks. Analysis was performed using NONMEM 7.4.1.

Results. Forty-three subjects with 1642 observations were available for analysis. Total and unbound MPA concentration data were fit to a two-compartment pharmacokinetic model with dual zero and first order absorption and lag-time, and first order elimination with allometric scaling.

Discussion. A population PK model has been developed from an Australian patient cohort, using joint total and unbound MPA concentrations and dual absorption to describe multiple concentration peaks. This could be used for dose optimisation to a target unbound MPA $AUC_{0\text{-}12}$, both peri-transplant and in the initial phase, where plasma protein binding changes alter the relationship between total and unbound exposure.

Woillard JB et al (2018) Clin Pharmacokinet 57(9):1211-1213
De Winter BC et al (2009) J Pharmacokinet Pharmacodyn 36(6):541-64
Nowak I et al (1995) Clin Chem 41(7):1011-7.

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A population pharmacokinetic model for metformin in patients receiving intermittent haemodialysis

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Introduction. Metformin is a first line treatment for type 2 diabetes mellitus. Metformin use in patients with renal impairment is limited due to the concern that elevated plasma concentrations may increase the risk of lactic acidosis. Dose reduction and metformin plasma concentration monitoring to ensure that concentrations do not exceed the proposed upper limit for safety of 5mg/L may mitigate the risk of lactic acidosis.

Aims. (1) To develop a population pharmacokinetic (PK) model for metformin in haemodialysis patients. (2) To predict doses that will maintain pre- and post-dialysis metformin plasma concentrations below 5 mg/L.

Methods. Data were sourced from four studies; one study included n=5 patients undergoing intermittent haemodialysis thrice weekly, and the remaining three studies had n=55 non-haemodialysis patients. Metformin data from non-haemodialysis patients were included to characterise the non-dialytic PK of metformin. The haemodialysis clearance of metformin was estimated using an additional clearance parameter. A population PK analysis was conducted using NONMEM® v.7.3. Stochastic simulations were conducted in MATLAB (2018a) to predict the probability of maintaining pre- and post-dialysis concentrations <5mg/L. Doses of 250, 500, 850, and 1000mg were simulated under two scenarios; 1) dosed after each haemodialysis session, and 2) dosed once daily.

Results. A total of 598 metformin plasma concentrations were available for analysis, including 172 from 5 haemodialysis patients. A two compartment PK model with first order absorption and elimination provided the best fit to the metformin data. The probability of pre-dialysis metformin concentrations exceeding 5mg/L was 0, 0.3, 10, and, 17% for 250, 500, 850, and 1000mg doses post-dialysis (thrice weekly) respectively and 2, 39, 76, and, 83% for 250, 500, 850, and 1000mg daily doses respectively.

Discussion. The model predicted that metformin plasma concentrations pre- and post-dialysis would rarely exceed 5mg/L for doses of 250-500mg post-dialysis, and 250mg daily.

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External evaluation of published population pharmacokinetic models of tacrolimus in adult heart transplant recipients

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Introduction. Numerous population pharmacokinetic (popPK) models of tacrolimus (TAC) in adult transplant recipients have been published. However, data on their implementation into clinical practice, the accuracy of Bayesian forecasting with concomitant or cessation of azole therapy or extrapolation to other transplant cohorts are scarce.

Aims. To externally validate the predictive performance of popPK models of TAC in adult heart transplant (HTX) recipients following the first 4 dosing occasions immediately post-HTX with concurrent azole therapy (Phase 1) and 4 dosing occasions after the cessation of azole therapy (Phase 2).

Methods. Published popPK models of TAC (n=59) were identified and a subset was selected based on specific criteria. Models were transcribed and predictions performed in NONMEM v7.4. Data from 40 HTX recipients (1735 concentrations) in 2017 treated with TAC at St Vincent's Hospital, Sydney were obtained immediately post-HTX up to 3 months post-azole cessation. Bayesian forecasting was used to establish the predictive performance (bias [median prediction error] and precision [median absolute prediction error]) of the models to predict TAC concentrations up to 4 dosing occasions in each phase. Clinically acceptable bias was between ±20% and precision was ≤20%.

Results. Of the 13 models evaluated, the model by Monchaud *et al*., displayed the best predictive performance with a bias of -2.1% and precision of 7.6% in Phase 1 (546 concentrations). However, all models were unsatisfactory in predicting TAC concentrations in Phase 2 (98 concentrations). In comparison to *a priori* predictions, the inclusion of concentrations improved model performance.

Discussion. The predictive performance of popPK models for TAC in post-HTX recipients varied substantially. The incorporation of azole therapy as a covariate may improve the accuracy of Bayesian forecasting. The applicability of extrapolating popPK models between different solid organ transplant populations warrants further investigation.

Monchaud *et al* (2012) Clin Pharmacokinet 51(3), 175-186.

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Systematic literature review comparing target concentration intervention to therapeutic drug monitoring of vancomycin therapy.

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Introduction. Dosing using the target concentration intervention (TCI) strategy overcomes many of the disadvantages associated with therapeutic drug monitoring (TDM).[1] However, TCI is implemented less widely in clinical practice despite supporting evidence for its superiority.[2] Vancomycin remains central to treatment of methicillin-resistant *Staphylococcus aureus*. TDM has been advocated as an essential component of optimal vancomycin therapy[3], however, the role of TCI is unclear.

Aim. To systematically review the literature comparing TCI and TDM strategies for vancomycin.

Methods. Pubmed was searched up to 2 August 2019 to identify publications comparing vancomycin concentration controlled dosing. Publications were eligible for inclusion if vancomycin dosing was guided by a measure of exposure (e.g. concentration or area under the curve) and a comparison between TCI and TDM dosing strategies was performed.

Results. Three publications[4-6] were eligible for inclusion. All studies were observational in nature and TCI was implemented as part of a change in practice. In all three studies TCI achieved a higher proportion of therapeutic exposures compared to TDM; TCI was also associated with a lower frequency of nephrotoxicity compared to TDM. Cost-effectiveness was not evaluated in any of the publications reviewed.

Discussion. The review supports the TCI approach to vancomycin individualisation over the TDM approach. There remains a paucity of prospective or experimental evidence supporting TCI for vancomycin, particularly when compared to the abundance of evidence from studies using a retrospective design. The optimal target exposure value, and method for linking dose to exposure remain uncertain and require further research.

1. Holford NH. Br J Clin Pharmacol. 1999 Jul;48(1):9-13. 2. Metz DK et. al. Optimising mycophenolic acid exposure in kidney transplant recipients: time for target concentration intervention. Transplantation. 2019;Online First Accepted. 3. Rybak MJ et. al. Clin Infect Dis. 2009 Aug 1;49(3):325-7. 4. Truong J et. al. J Clin Pharmacol. 2018 Sep;58(9):1123-1130. 5. Wong T et al. Pharmacotherapy. 2019 Apr;39(4):433-442. 6. Neely MN et. al. Antimicrob Agents Chemother. 2018 Jan 25;62(2). 6

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Assessing the accuracy of two Bayesian forecasting programs in estimating vancomycin drug exposure

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Introduction. Current therapeutic drug monitoring guidelines for intravenous vancomycin in adults identify drug exposure (area-under-the-curve, AUC) as the best pharmacokinetic (PK) indicator of therapeutic outcome.

Aims. To assess the accuracy of two Bayesian forecasting programs in estimating vancomycin $AUC_{0-\infty}$ in adults with limited blood concentration sampling.

Methods. The application of a 1-compartment and six 2-compartment vancomycin population PK models in two Bayesian forecasting programs was examined in a retrospective cohort of non-obese adult subjects (n = 22) with stable renal function. Patients were intensively sampled following a single 1000 mg dose (if weight < 50 kg, 15 mg/kg) infused over at least 40 min. An individual's $AUC_{0-\infty}$ was calculated by fitting all vancomycin concentrations to a 2-compartment model using weighted, nonlinear least-squares regression (defined as AUC_{TRUE}). AUC_{TRUE} was then compared to the Bayesian-estimated $AUC_{0-\infty}$ values using a single vancomycin concentration sampled at various times post-infusion. Accuracy was quantified using bias (mean prediction error) and imprecision (mean absolute prediction error). The program was deemed "unbiased" if the confidence intervals of bias contained zero and "precise" if imprecision was <20%.

Results. Optimal sampling times varied across different models. $AUC_{0-\infty}$ was generally over-estimated at earlier sampling times and under-estimated at sampling times greater than 4 h post-infusion. The most accurate models by Goti et al. (2018) and Thomson et al. (2009) had unbiased and precise sampling times between 1.5 – 6 h and 0.75 – 2 h post-infusion, respectively. Precise but biased sampling times for Thomson et al. were between 4 and 6 h post-infusion.

Discussion. Certain 2-compartment vancomycin population PK models allow for accurate Bayesian estimation of the $AUC_{0-\infty}$ using a single concentration. This study established the optimal blood sampling time to estimate vancomycin drug exposure in order to optimise successful therapeutic outcomes.

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Regulatory Pharmacometrics in Australia

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Introduction. Pharmacometrics (PMx) is an important tool used by sponsors to support regulatory submissions. TGA is increasingly utilising PMx in regulatory decision making through re-analysis and expert working group (WG) input.

Aims. To report on the internal referrals for PMx evaluation at TGA and assess the utility of PMx in supporting regulatory decisions such as product information approval.

Methods. PMx referrals from within the TGA during January 2017 and December 2018 were reviewed. They were categorised based on the type of PMx evaluation e.g. PopPK, PK/PD, PBPK etc. and how PMx were utilised to support the submission. A PMx analysis was considered to be significant if the decision was influenced by the submitted PMx analyses i.e. effectiveness and safety, or selection of the dosing regimen. For product information, PMx analysis was considered significant if the results supported the clinical pharmacology, dosage and administration, or safety (e.g. warnings and precautions, or contraindications) sections.

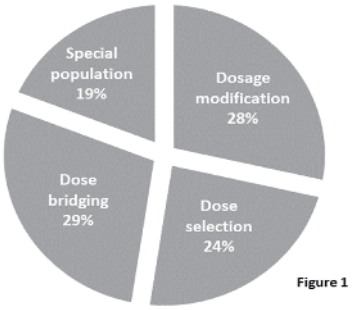

Figure 1

Results. A total of 46 referrals were received. There were 43 PopPK analyses, 26 PK/PD analyses, 4 PBPK analyses and 1 simulation analysis in the 46 referrals. 28 referrals were critically evaluated (re-analysis and/or WG input). The remaining (18) either had other agency evaluation reports (6), were minor extensions to the previous evaluations (8), or did not have significant input into submission (4). Of the 21 analyses which were informative, Figure 1 describes which aspects of the regulatory submission were supported by the PMx analyses.

Discussion. This analysis provides an overview of the impact of PMx on regulatory submissions in Australia. It also informs the areas to be targeted for PMx evaluations to maximise contribution to the decision-making process.

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Optimising cyclic GMP signalling for the treatment of pulmonary hypertension

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Introduction. Pulmonary hypertension (PH) is a debilitating, incurable disorder characterized by increased pulmonary vascular resistance (PVR) and a degenerative remodelling of the pulmonary arterial tree, leading to right ventricular (RV) failure; new medicines are desperately required.

Aims. Therapeutics promoting cyclic GMP (cGMP) signalling, for example phosphodiesterase 5 inhibitors (PDE5i) and soluble guanylyl cyclase (NO-sensitive GC-1 & GC-2; 'sGC') stimulators, are clinically-effective but only slow disease progression rather than offering resolution. We hypothesised that by targeting multiple aspects of cGMP bioactivity concurrently it might be possible to further enhance therapeutic benefit.

Results. Pre-clinical studies demonstrated in pulmonary vessels *in vitro*, in the pulmonary vasculature *in vivo*, in pulmonary artery smooth muscle cells from PH patients, and in experimental models of PH, that manipulation of natriuretic peptide bioactivity and activation of cognate receptor GC-A is an efficacious strategy for stimulating cGMP-signalling, targeting the pulmonary vascular, and reversing both the structural and haemodynamic changes that characterise the disease. This can be readily achieved via combination of a PDE5i with a neprilysin (NEP; enzyme that hydrolyses & inactivates natriuretic peptides) inhibitor. In accord, in a proof-of-concept Phase IIa clinical trial (COMbination therapy in PAH with RacEcadotril; COMPARE) such a dual therapy is effective in patients with pulmonary arterial hypertension, augmenting natriuretic peptide bioactivity and reducing pulmonary artery pressure (without significantly affecting systemic blood pressure).

Discussion. This translational project supports the repurposing of NEP inhibitors for PH (in combination with existing PDE5i therapy) and thereby holds potential as a significant, cost-effective therapeutic advance for PH and related pulmonary disorders.

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Pharmacological therapies to target reactivity and remodelling in cardiopulmonary disease

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The pathogenesis of asthma and pulmonary hypertension (PH) clearly differs, with asthma affecting the airways and pulmonary hypertension affecting the vasculature. Bronchopulmonary dysplasia (BPD), a devastating disease affecting premature babies affects both, with impaired lung development and reduced pulmonary vascularity increasing the risk of the development of asthma and pulmonary hypertension. Excessive smooth contraction and dysregulated remodelling are common features of these cardiopulmonary diseases, and remain areas of unmet medical need. Current drugs that relax airways and pulmonary arteries have reduced efficacy with increasing disease severity, while there are no effective treatments to reverse progressive fibrosis, or to restore normal vascular development in BPD.

This presentation will outline emerging preclinical evidence identifying novel therapeutics with the potential to reduce patient symptoms and/or limit disease progression in asthma, PH and BPD. For asthma, the potential benefits of FFA1 and FFA4 agonists, CaSR negative allosteric modulators (NAMs) and relaxin will be described. These agents have dilator efficacy under conditions of reduced responsiveness to β_2-adrenoceptor agonists, while relaxin also reverses established fibrosis in a mouse model of chronic allergic airways disease that mimics key features of asthma (Lam et al., 2018). For PH, Annexin-A1 mimetics have shown potential as novel dilator therapies with greater potency and efficacy than sildenafil in mouse pulmonary arteries. Finally, in an LPS/hyperoxia model of BPD, treatment of newborn mice with the interleukin-1 receptor antagonist anakinra reduced airway remodelling but not airways hyperresponsiveness (Royce et al., 2016). Critically, the beneficial effects of anakinra on alveolar and pulmonary vascular development were protective against the development of pulmonary hypertension (Bui et al., 2019). These novel pharmacological therapies offer promise for improved patient outcomes in asthma, PH and BPD.

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Lam et al., Serelaxin as a novel therapeutic opposing fibrosis and contraction in lung diseases. Pharmacol Ther. 2018;187:61-70.
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Novel therapeutic strategies for the treatment of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) encompasses a group of lung diseases that progress to emphysema, chronic bronchitis and refractory asthma. These diseases are characterised by lung inflammation, persistent airflow limitation and a progressive decline in lung function. In severe cases, COPD can also lead to hypoxia and right-sided heart failure. Despite the significant burden that these diseases represent, currently-available therapies only offer symptomatic management of disease progression through their ability to reduce lung inflammation (inhaled corticosteroids) and/or relax the muscles around airways (bronchodilators). Major components of disease pathology that are left untreated are the structural changes that occur in the damaged lung that include airway epithelial damage and fibrosis, which significantly contribute to the severity of airway obstruction. This presentation will provide an overview of COPD progression, with a particular emphasis of the factors that contribute to airway remodelling-induced fibrosis progression. It will also discuss some recent findings obtained on the pre-clinical evaluation of novel epithelial cell repair compounds that were developed by AusBio Ltd, in a murine model of cigarette smoke-induced COPD.

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Improving immunotherapy response outcomes for lung cancer patients by developing novel biomarker assays

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Introduction. Lung cancer is the leading cause of cancer related deaths, accounting for over 1.8 million deaths annually on a global scale. The overall survival rate is less than 15% over 5 years, which reflects typical late stage diagnosis of the disease and a paucity of effective therapies. In very recent times, immunotherapy has shown remarkable curative responses in about 20-30% advanced lung cancer patients. However, the cost of monoclonal therapy is significant, and the treatment is not without adverse side effects including dermatologic, gastrointestinal, hepatic and endocrine events.

Aims. To improve outcomes for lung cancer patients by developing better biomarkers that are predictive of response to immunotherapy.

Methods. Tumour biopsies obtained from non-small cell lung cancer (NSCLC) patients (n = 48 adenocarcinoma and n = 40 squamous cell carcinoma) and control lung biopsy specimens (n = 20) were obtained from the Victorian Cancer Biobank. Biomarkers including MMP9, TIMP3 and PD-L1 transcript copy numbers were determined within a single assay by multiplex digital droplet ddPCR using Taqman primers and the QX200 Droplet Digital PCR System.

Results. Using our optimised triplex ddPCR assay, the MMP9:TIMP3 ratio was significantly elevated in NSCLC biopsies and using a cut-off of >0.028, was 99% (95% CI; 80.5-94.5) sensitive and 80% specific for identifying malignant biopsies. The PD-L1:TIMP3 ratio significantly associated with PD-L1 tumour cell immunohistochemistry staining ($r = 0.539$, $p < 0.0001$) and was significantly higher in biopsies with >50% PD-L1 tumour cell staining ($p < 0.0001$).

Discussion. In summary, we have developed a new clinical workflow that can accurately quantify PD-L1 tumour levels using ddPCR and our approach provides sufficient nucleic acid for screening additional targetable mutations such as EGFR, ALK and ROS1 from a single small biopsy. The major advantages of our ddPCR assay is that it is highly sensitive, thereby potentially avoiding the need for re-biopsy and can be fully automated. Future studies will need to determine diagnostic ddPCR values that are predictive of clinical response to PD-1/PD-L1 immunotherapy.

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Complexity and clinical implications of multiple chronic conditions and polypharmacy in the older population

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Care of the older population with multiple chronic conditions is complex due to the associated polypharmacy, drug interactions, treatment conflicts and competing health priorities, that can potentially place patients at increased risk of medication-related harms. Innovative methods to assess the effectiveness and safety of complex medicine regimens in this heterogeneous population are required to provide the evidence that is currently lacking to guide treatment decisions. This first part of the symposium will highlight the complexities associated with care of older people with multiple chronic conditions and polypharmacy, followed by discussion of potential methodological strategies to advance the evidence-base. A multifactorial approach is needed that includes in part, assessment of universal health outcomes that are important to the older patient which includes overall benefits and harms, rather than traditional disease-specific health outcomes. In addition, consideration and reporting of effect modifiers such as age, frailty status, comorbidity and polypharmacy, including individual medications are needed. This will provide quantification of outcomes for specific patient subgroups and a step towards personalising treatments to ultimately improve health and quality of life for the older population.

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A multi-country network to optimise medicine use in people with Dementia: The NeuroGEN

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People with dementia are often excluded from randomised controlled trial (RCTs). This means that evidence for medication safety and efficacy is lacking. This is despite that people with dementia have multiple co-morbid conditions and use multiple medications. Increases in the availability of administrative data have led to increased opportunities for big data research in pharmacoepidemiology, particularly in the patient groups not represented in RCTs. The NeuroGEN (Neurodegenerative diseases Global Epidemiology Network) aims to investigate medication safety and effectiveness in people with neurodegenerative diseases such as dementia in a globally representative population. NeuroGEN includes to-date 9 counties or geographical regions representing their >100 million inhabitants. In 2018, >30 researchers across these countries met in Hong Kong to explore data availability and identify potential joint research projects. The plans were further developed in the 2nd meeting in London, August 2019. Funding has been secured to the first projects on the effect of guideline-recommended medication use for chronic comorbidities in people with dementia.

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Methodological challenges and innovative solutions: Understanding the complexity of pharmacoepidemiological studies in the older population

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Observational studies can complement evidence from RCTs, in particular when studying (rare) adverse events, long-term adverse events, and potentially contribute evidence on effectiveness under 'Real World' circumstances. The main limitation of observational studies is the lack of randomisation and blinding. In particular the study of intended effects can be biased due to confounding by indication. In the elderly, frailty adds an additional complexity to the design and analysis of observational studies. Frailty may be important reason to withhold preventive treatments such as statins, antihypertensives, and/or flu vaccines in those with a poor prognosis therefore high risk of mortality. Furthermore, those at high risk of death are more likely to be hospitalised in the period closer to death. In primary care database this may result in an immeasurable time bias due to the fact that patients are not able to get their prescription or dispensing of medication. Both mechanisms may make users of drugs look good compared to non-users and introduce confounding and information bias. In this presentation several strategies will be discussed such as active comparator designs and propensity score analysis to prevent and control confounding in observational studies of drug effects in the elderly.

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Clinical decision support tools for prescribing in the older population: from qualitative dashboards to quantitative modelling and simulation to enable precision dosing

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Older patients are particularly susceptible to adverse drug reactions (ADRs) for several reasons. (1) The evidence base for drug therapy is often absent or weak. (2) Co-existing medical conditions are treated independently based on specialist guidelines, resulting in inappropriate polypharmacy and increased rates of adverse drug-drug interactions. (3) Various pharmacokinetic (PK) and pharmacodynamic (PD) changes occur with normal aging and with disease, including impaired renal and hepatic function, and lower physiological "reserve". Precision dosing is defined as correct dose selection by a prescriber for an individual patient at a given time (Polasek et al. 2018). This presentation will review the clinical decision support tools (DSTs) available currently for precision dosing in the elderly, including prescribing modules in electronic health records (EHRs) that provide advice, link to commercial and independent drug information (e.g., eMIMS, eAMH, eTGs), and check prescriptions. More sophisticated quantitative approaches as DSTs will be then addressed, focussing on "model-informed precision dosing (MIPD)" – population PK/PD models linked to biomarkers of drug effects, physiologically based PK models, quantitative systems pharmacology and toxicology (QSP/QST) models, and artificial intelligence/machine learning-derived algorithms based on real world evidence of clinical- and patient-reported outcomes. Model-informed DSTs for precision dosing can interface with prescribers as stand-alone products (application or cloud-based) and/or by integration into EHRs. As with all novel technologies in clinical medicine, the value of MIPD will be decided ultimately by the extent to which it can improve cost-effective patient care.

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Proximal study of biased agonismat G protein-coupled receptors

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Introduction. Biased agonism is now a well-accepted concept in G protein-coupled receptor (GPCR) research and is recognized as an avenue for developing novel therapeutic strategies. Although many studies have focused on investigating biased behavior of GPCRs, little is known regarding the direct involvement and the influence of proximal binding partners such as different transducers and receptor dimers. The mechanistic basis of how differences in ligand-receptor interaction lead to distinct effector engagement and how this in turn contributes to distinct cellular signalling profiles is also unclear. Here, we have ultilised the cognate ligand GLP-1 and other peptide or non-peptide ligand with distinct binding kinetics, and assessed the differential engagement with signalling and regulatory transducers. The effects of homodimerization are also examined.

Aims. Investigating the contribution of receptor proximal binding partner on GLP-1R biased agonism.

Methods. Ligand binding kinetics was measured by NanoBRET technology using plasma membrane purified from HEK293A cells overexpressing Nluc-hGLP-1R. Ligand induced G protein activation was measured using NanoBIT luciferase complementation assay. Second messengers and receptor trafficking were measured as mentioned previously.

Results. Our results suggest that the kinetics of ligand-receptor engagement directly regulates the efficiency of receptor-G protein coupling. In addition, homodimersiation of GLP-1R has distinct role in regulating GLP-1R signalling and regulation in a ligand dependent manner.

Conclusion. This study furthers our understanding of the molecular events that affect ligand binding to intracellular signalling.

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Unravelling the molecular basis of biased agonism using cryo-EM

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G protein-coupled receptors (GPCRs) are the largest family of cell surface drug targets. Consequently, there is high interest in understanding the structure of members of this receptor superfamily and molecular detail of how ligands and transducer proteins interact with them. Our laboratory has been applying single particle cryo-EM to determination of active GPCR structures, using minimally modified receptors. Our work has been principally focused on the class B GPCR subfamily that bind large peptide hormones and are well established clinical targets for the treatment of major disease, including migraine, irritable bowel syndrome, diabetes, obesity and neurodegeneration. We have now solved structures of multiple different receptors, providing wide structural coverage of the major subfamilies of class B GPCRs. Included within this are structures of the same receptor bound to native peptide agonists, biased peptide agonists and non-peptide agonists. In combination with molecular pharmacology and molecular dynamics simulations, we are gaining substantial insights into diverse modes of ligand binding and receptor activation that lead to G protein coupling and downstream signalling.

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Structural basis for biased agonist action at the angiotensin II type 1 receptor

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"Biased" agonists of G protein-coupled receptors (GPCRs) preferentially activate a subset of downstream signaling pathways. Using a synthetic camelid antibody fragment crystallization chaperone we were able to determine crystal structures of the human angiotensin II type 1 receptor (AT1R) bound to three ligands with divergent bias profiles: the balanced endogenous agonist angiotensin II (AngII) and two strongly β-arrestin-biased analogs. Compared to other ligands, AngII promotes more substantial rearrangements not only deep within the ligand-binding pocket but also in a key polar network in the receptor core, which in most GPCRs forms a sodium-binding site. Divergences from the family consensus sequence in this region may predispose it to act as a biased signaling switch in the AT1R and certain other GPCRs (e.g., chemokine receptors), enabling these receptors to adopt conformations capable of activating β-arrestin but not G protein signaling.

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Structural and functional insights into GPCR-β-arrestin interaction and signaling

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Our research program is focused on understanding the largest class of cell surface proteins in our body which are referred to as G protein-coupled receptors (GPCRs). These receptors are intricately involved in almost every physiological process and approximately one third of the currently prescribed medicines exert their therapeutic effects through these receptors. The overarching theme in my laboratory is to understand the structure, function and regulation of GPCRs, and leverage this information to design and discover novel therapeutics with minimal side-effects. I shall present our recent work on understanding the conformational complexity of GPCR-β-arrestin interaction and how structural differences in receptor-β-arrestin complexes orchestrate distinct functional outcomes.

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Synthesis of novel P2X1-purinoceptor antagonists for use in male contraception

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Introduction. While present contraceptive methods are effective, there is clearly a need to develop additional methods of contraception for males, a market which is clearly lacking. Therapeutic targets for male contraception are associated with numerous problems due to their focus on disrupting spermatogenesis or hormonal mechanisms to produce dysfunctional sperm. We have previously described an autonomic male contraceptive strategy through the dual genetic deletion of α_{1A}-adrenoceptors and P2X1-purinoceptors in male mice thereby blocking sympathetically mediated sperm transport through the vas deferens during the emission phase of ejaculation (White et al 2013). Since there are already several suitable available α_{1A}-adrenoceptor antagonists but as yet no suitable P2X1-purinoceptor antagonists, the next logical incremental step towards the development of our male contraceptive is to produce an appropriate P2X1-purinoceptor antagonist for use in combination with an α_{1A}-adrenoceptor antagonist.

Aims. To synthesize a suitable P2X1-purinoceptor antagonist tool compound that is potent, efficacious and safe for use in proof of principle experiments that pharmacological blockade of α_{1A}-adrenoceptors and P2X1-purinoceptors produces male contraception.

Methods. Synthesized compounds were tested for initial activity using electrically field stimulated *(parameters: 0.5 ms pulse duration, 60 V, 2 Hz)* preparations of isolated rat vas deferens. Antagonism of active compounds (30-100 µM) was assessed against discrete concentration-response curves to exogenous administration of αβmethylene ATP on unstimulated preparations of isolated rat vas deferens. Activity of novel compounds was also assessed by inhibition of αβmethylene ATP induced intracellular Ca^{2+} mobilisation in HEK293A cells expressing P2X1-purinoceptors.

Results. 80 novel compounds were synthesized and tested with 5 yielding IC_{50} values of < 30 µM against electrical field stimulation induced contractions of isolated rat vas deferens. Active compounds displayed non-competitive anatagonism against P2X1-purinoceptors but not cholinergic or adrenergic receptors.

Discussion. MIPS0020912 was the most active compound with an IC_{50} of 14.0 (95% C.L.: 12-16) µM.

White CW et al (2005) Proc Natl Acad Sci USA 163: 891-907

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Exploring allosteric modulation of GPCRs for the treatment of gastrointestinal disorders.

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Introduction. Allosteric modulation is a therapeutically important conceptual advancement in the G protein-coupled receptor (GPCR) field. To date, allosteric modulation in the context of the enteric nervous system (ENS) and as a potential pharmacological approach for treating gastrointestinal (GI) disorders is largely unexplored.

Aims. To examine allosteric modulation in the ENS using the delta opioid receptor (DOR) as a model GPCR, and to determine whether allosteric modulation is a suitable approach for treating GI symptoms in preclinical models of irritable bowel syndrome (IBS).

Methods. The DOR positive allosteric modulator (PAM) BMS-986187 was comprehensively characterized in model cells and in the ENS by pharmacological analyses of neurogenic contractions of the mouse colon and by DOR internalization assays. The ability of BMS-986187 to influence GI motility was examined both *in vitro* and *in vivo*.

Results. BMS-986187 exhibited probe dependence and selectivity for DOR in the neurogenic contraction assay. In addition, BMS-986187 enhanced the ability of the endogenous DOR agonist Leu-enkephalin to inhibit electrically-stimulated contractions. BMS-986187 augmented both the internalizing properties of the DOR agonist ARM390 and reflex-evoked DOR endocytosis, demonstrating modulation at the neuronal level. BMS-986187 alone reduced the generation of motor patterns in the isolated colon, confirming that DOR-expressing myenteric neurons influence motility. Finally, BMS-986187 reduced fecal output and diarrhea onset in the novel environment stress and castor oil models of IBS, respectively.

Discussion. Collectively, we have provided evidence that BMS-986187 enhances DOR-mediated responses in the ENS and ameliorates motility disturbances in preclinical models of IBS. This study supports allosteric modulation of GPCRs as a therapeutic approach for treating GI dysmotility.

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Purinergic P2X7 receptor inhibition protects urothelial cells from acrolein-induced cell death, which is independent of oxidative stress

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Introduction. Acrolein is an unsaturated aldehyde with high toxicity index, which can cause severe damage to cells through a variety of mechanisms, including the induction of oxidative stress leading to apoptosis and cell death. It is known that the purinergic P2X7 receptor (P2X7R) plays an important role in apoptosis. We have recently reported that inhibition of P2X7R protected against acrolein-induced apoptosis in the porcine bladder (Taidi et al., 2018).

Aims. The current study aimed at exploring the effects of acrolein on oxidative stress and cytotoxicity in urothelial cells, and determining whether the blockade of P2X7R could attenuate acrolein induced cell damage.

Methods. Urothelial cells were isolated from the bladder of female porcine (n=6). Cells were plated and cultured till confluent, and then treated with different concentrations of acrolein and/or the selective P2X7R antagonist A804598. For the cell viability assay, 10% resazurin dye was added to each well, and the fluorescence signal was read by a plate reader. To determine the oxidative stress, the cell permeant reagent 2',7' –dichlorofluorescin diacetate, a fluorogenic dye, was applied to measure the production of reactive oxygen species (ROS) within the cells.

Results. Acrolein (12.5 - 100 μM) markedly reduced the urothelial cell viability and increased ROS production in a concentration-dependent manner. The cytotoxic effect of acrolein (50 μM) was slightly but significantly inhibited by the pre-treatment of cells with A804598 at 1 μM ($P < 0.05$, two-way ANOVA), and was completely reversed to the control level by the application of A804598 at 10 μM ($P < 0.001$). Nevertheless, the enhanced ROS production by acrolein (50 μM) treatment was not affected by the pre-incubation of cells with A804598 (up to 100 μM). Other selective P2X7R antagonists AZ10606120 and A438079 also showed no effect on acrolein induced ROS production.

Discussion. In this study, we have demonstrated that acrolein causes strong cytotoxicity and ROS production in porcine primary urothelial cells. P2X7R inhibition can protect cells from acrolein-induced cell death, but not from acrolein-induced oxidative damage. Other mechanisms may be involved in the protective effect of P2X7R inhibition.

Taidi Z, Mansfield KJ, Moore KH, Liu L (2018). Neurourol Urodyn 37: S104-S106.

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Age-related changes in histamine receptor mediated contractions of the urinary bladder

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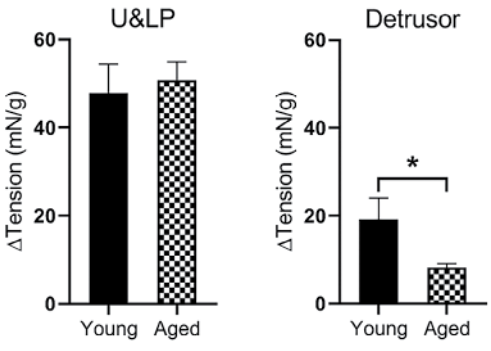

Introduction. Pro-inflammatory mediators may have a role in various bladder contractile disorders, such as overactive bladder. Histamine is known to induce significant increases in both the tension and frequency of spontaneous phasic contractions in both urothelium with lamina propria and detrusor muscle via the activation of H1 receptor (Stromberga et al, 2019). However, it is unclear how age affects these contractile responses to histamine. Aims. This study aimed to compare urothelium with lamina propria (U&LP) and detrusor responses to histamine in young and aged tissue samples. Methods. Adjacent strips of porcine U&LP and detrusor obtained from young and aged animals were mounted in a gassed Krebs-bicarbonate solution and responses to histamine obtained in the absence and presence of selective antagonists. Data analyses were performed using Student's *t*-tests. This research was supported by Australian Bladder Foundation. Results. Treatment with histamine (100 μM) in U&LP of young animals caused increases in baseline tension by 47.84±6.52 mN/g ($p<0.001$, n=51) and by 50.76±4.10 mN/g ($p<0.001$, n=55) in aged animals. Furthermore, the frequency of spontaneous phasic contractions was significantly enhanced in response to histamine in both young and aged ($p<0.001$ for both age groups). In detrusor, young tissues showed a significantly ($p<0.05$) higher increase in baseline tension of 19.10±4.92 mN/g (n=51) when compared to aged tissues exhibiting increases of 8.21±0.89 mN/g (n=56) in response to histamine (100 μM). Treatment with H2 agonist in U&LP of young animals decreased baseline tension by 13.97±3.45 mN/g (n=12, $p<0.05$) but had no effect in aged tissues and in detrusor preparations of both young and aged. Inhibition of H1 receptors resulted in significantly ($p<0.05$) reduced contractile responses to histamine in both young and aged animals. Antagonism of H3/H4 had no effect on contractions in both young and aged. Discussion. The histamine receptor system may play an important role in the maintenance of bladder function, or in the stimulation of some contractile disease states in both young and aged tissues.

Stromberga Z et al (2019) Sci Rep 9:1-7. https://doi.org/10.1038/s41598-019-40384-1

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The impact of ageing on urinary bladder muscarinic receptor activity

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Introduction. Of all patients prescribed antimuscarinic therapy for overactive bladder, those aged under 60 years are more likely to discontinue treatment earlier than their older counterparts. The cause of this reduced adherence to treatment regimens remains unclear, however may be attributed to either lifestyle changes or age-related physiological changes. Aims. This project aimed to investigate the influence of ageing on contractile activity of the urinary bladder urothelium with lamina propria (U&LP), and detrusor tissue layers in response to clinically prescribed antimuscarinics. Methods. Porcine U&LP or detrusor strips from either young or old pigs were mounted in gassed Krebs-bicarbonate solution at 37°C and carbachol concentration-response curves recorded in the presence and absence of selective muscarinic antagonists (1μM for all antagonists). pEC50 values for each curve were obtained and the differences between young and old tissues were analysed using ANOVA (multiple comparisons) with Dunnett's post-test ($p<0.05$ being significant). Results. In aged U&LP, carbachol exhibited contractions 40.12±4% higher when compared to responses in younger bladders ($p<0.01$, $n=32$). In responses to carbachol, tolterodine produced parallel right-shift curves from the control, with an estimated pKD of 7.56 in young U&LP ($n=8$) and 8.14 in aged ($n=8$, $p<0.05$). Tolterodine also inhibited detrusor responses, with an estimated pKD of 7.52 in young ($n=8$) and 8.07 in aged ($n=8$, $p<0.05$). This demonstrated that tolterodine had a greater effect inhibiting bladder contractions from aged animals. Oxybutynin's estimated pKD in young U&LP was 7.81 ($n=7$), yet 8.48 in aged ($n=8$), demonstrating a significantly greater inhibition in older tissues ($p<0.05$). Oxybutynin also demonstrated increased inhibition in the detrusor, with an estimated pKD 8.52 in young ($n=8$) and 7.95 in aged ($n=8$, $p<0.05$). Oxybutynin also inhibited the maximum contraction to carbachol. Solifenacin and darifenacin equally inhibited the contraction to carbachol in U&LP and detrusor, and their effectiveness was not influenced by age. Discussion. All antimuscarinics examined inhibited contractile responses to carbachol, with tolterodine and oxybutynin having increased inhibition in old tissues compared to young tissues. This suggests that the observed increased persistence for overactive bladder treatment regimes in older adults may be, in some cases, attributed to a heightened effectiveness of antimuscarinic therapy.

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Erianin induces apoptosis and autophagy in human prostate cancer cell

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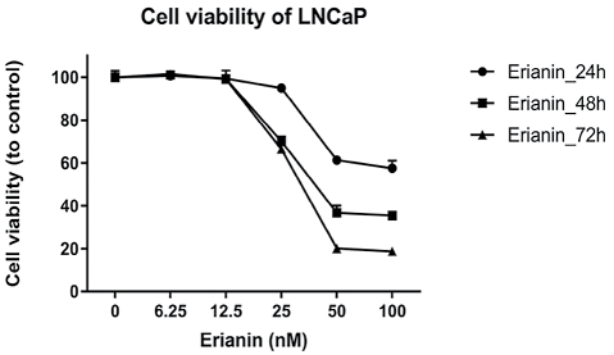
Introduction. Erianin is a bibenzyl derivate found in *Dendrobium chrysotoxum*. It has similar structure to combrestatin which displays potent anticancer activity. Several studies have shown its anti-cancer properties against cancer cells including, osteosarcoma, nasopharyngeal carcinoma, breast cancer and leukemia. To our knowledge, its anti-cancer activity has not yet been reported in prostate cancer.



Aims. To explore the anti-cancer activity of erianin in prostate cancer cells.

Methods. Cell viability was determined using MTS assay. Cells were treated with various concentration of erianin (0-100nM) for 24, 48 and 72h. Cell lysates were prepared and analysed by western blotting for Bcl-2 family and caspase-3.

Results. Erianin inhibited cell viability of LNCaP cells in a dose and time-dependent manner. This supports its inhibition effect on cell colony growth. Erianin induced apoptosis by upregulating Bcl-2 propapoptosis protein (Bak), BH3 only (Bid), suppressing antiapoptosis proteins (Bcl2 and Mcl1) and increasing cleaved caspase-3. Erianin induced autophagy in LNCaP cells. Treatment with bafilomycin A1, an autophagy inhibitor, increased erianin-induced apoptosis which indicating the contribution of erianin to the cell survival in the autophagy process.

Discussion. Erianin demonstrated cytotoxic activity, induced apoptosis via the mitochondrial pathway and induced autophagy in LNCap cells. These results indicate that erianin has significant anti-cancer properties and is a promising drug candidate in prostate cancer therapy.

Wang H et al (2016) Cell Death Dis 7:1-12

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Efficacy of endothelin receptor antagonists in patients with the pulmonary arterial hypertension-connective tissue disease subtype: a systematic review and meta-analysis

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Introduction: Patients with connective tissue disease-pulmonary arterial hypertension (PAH-CTD) subtype have poorer outcomes compared to other PAH disease subtypes[1], therefore the identification of effective treatments is critical.

Aims. To evaluate the efficacy of endothelin receptor antagonists (ERAs) in PAH-CTD based on clinical failure composite end points (CFEs, primary end-point) and 6-min walk distance (secondary end-point), both individually and in combination with phosphodiesterase 5 inhibitors (PDE5is).

Methods: We conducted a systematic review, using MEDLINE, COCHRANE, CINAHL and EMBASE databases (from inception to May 2019), of randomised control studies assessing ERAs efficacy (mono-therapy or combination therapy). Studies were screened by 2 independent reviewers.

ANALYSIS	Clinical effectiveness measure	Confidence interval p value
ERA vs Placebo		
Clinical failure composite end point	OR 0.77	[0.50, 1.19] p=0.25
6 minute walk distance change from baseline	Mean difference +21.72m	[-16.01, 59.44] p=0.26
Combination vs Monotherapy		
Clinical failure composite end point	OR 0.54	[0.32, 0.90] p=0.02
6 minute walk distance change from baseline	Mean difference +15.39m	[7.55, 23.23] p=0.0001

Results: Final analysis included 1253 participants, across 11 studies. ERA monotherapy did not reduce CFE vs. placebo [odds ratio 0.77, 95% CI [0.50, 1.19] p=0.25). Combination therapy(ERA+PDE5i) significantly reduced CFE vs. monotherapy (odds ratio 0.54, 95% CI [0.32, 0.90], p=0.02); and increased 6 min walk distance (mean difference +15.39m, 95% CI [7.55, 23.23], p=0.0001).

Discussion: ERAs when used in combination with PDE5is, are associated with better clinical outcomes in PAH-CTD. Considerations should be made towards initial combination therapy as a standard of care.

[1]Coghlan, J. G. et al (2017). *Annals of the Rheumatic Diseases*, *76*(7), 1219–1227.

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Use of cannabinoids to manage behaviour in children with developmental/behavioural disorders

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Introduction. Disorders such as Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) represent at least 2/3 of outpatient paediatric consultations (Hiscock et al, 2017). Associated challenging behaviours often result in prescription of stimulants, sedatives and antipsychotics. Stories abound in the lay press and social media of symptom control using cannabinoids and some parents are using illicit cannabis products with associated risks of low-grade products and contaminants. Implications for the treating paediatrician are significant.

Aims. To review the evidence for cannabinoids to manage behavioural problems in children with ASD and ADHD and discuss the responsibilities of paediatricians with parents using illicit products.

Methods. Embase was searched using a variety of cannabis AND paediatric related Emtree terms. Results were screened to select studies pertaining to behaviour management in ASD and ADHD, excluding single case reports. A search was also undertaken of the ClinicalTrials.gov and the ANZCTR databases to identify studies.

Results. There are no published RCTs comparing cannabinoids with placebo or any other intervention. Several retrospective observational studies report improvement rates of 60-84%. A prospective observational study of transdermal cannabidiol in 20 children and adolescents with Fragile X syndrome showed a reduction in behavioural symptoms (Heussler et al, 2019). Several further prospective observational studies in other conditions and 4 RCTs are underway.

Discussion. Despite little evidence to recommend cannabinoids for treatment of behavioural problems in children and adolescents, some parents are treating children with illicit products. Further to meeting any relevant child protection legal obligations, the onus is on the clinician to ensure that parents are aware of potential risks. Further research to determine safety and explore potential comparative efficacy of cannabinoid treatment for this indication is required.

Heussler H, Cohen J, Silove N et al (2019) J NEURODEV DISORD
Hiscock H, Danchin MH, Efron D et al (2017) JPAEDIATR CHILD H 53:55-61

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Clinical outcomes and pharmacokinetic-pharmacodynamic target attainment in patients treated with oral flucloxacillin plus probenecid for staphylococcal infections

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Introduction. Oral flucloxacillin may be administered with the organic anion transporter inhibitor probenecid, to increase flucloxacillin concentrations, for the treatment of infections with gram positive organisms, including methicillin sensitive *Staphylococcus aureus* (MSSA). There are limited data regarding PK-PD target attainment and clinical outcomes in patients treated with this combination.

Aims. To describe i) PK-PD target attainment of free drug time above minimum inhibitory concentration, $fT_{>MIC}$, for an MIC_{90} = 0.5mg/L for MSSA, and ii) clinical outcomes in outpatients treated with oral flucloxacillin plus probenecid.

Methods. We performed a prospective, single-centre, observational study of adult patients (≥18 years) treated with oral flucloxacillin 1000 mg plus probenecid 500 mg 8-hourly (with food), as oral step-down, for proven or probable staphylococcal infections. Patients gave one mid-interval blood sample at steady-state, which was augmented with richly-sampled PK data from volunteers (n=11) for measurement of total and free flucloxacillin. Monolix software was used to develop a population pharmacokinetic model and estimate the PK-PD target attainment using $fT_{>MIC}$ of 30% and 50% for bacteriostatic and bactericidal activity respectively.

Results. The 45 patients (73% male) had a median (range) age of 49 years (20 – 74), weight of 90 kg (59 – 167) and BMI of 28 kg/m^2 (19 – 69). Median eGFR (CKD-EPI) was 110 mL/min (43 – 166). The most common infections were osteomyelitis (n=18, 40%) and septic arthritis (n=12, 27%). Intravenous treatment was given for a median of 26 days (1 – 73), and oral for 28 days (8 – 362). A $fT_{>MIC}$ of 0.5mg/L for 30% and 50% of the dosing interval was achieved by 90% and 50% of the patients, respectively. 91% (n=40) of patients were cured at 30 days following completion of therapy.

Discussion. Oral flucloxacillin plus probenecid following initial intravenous treatment was associated with a high cure rate, despite modest rates of attainment of pre-specified PK-PD targets.

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Polypharmacy in palliative care: a retrospective comparison of two explicit deprescribing tools

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Introduction. A major barrier to deprescribing inappropriate medicines in palliative care is lack of explicit guidance. Two explicit deprescribing tools have been published specifically for use in a palliative population, OncPal (Lindsay et al, 2015) and STOPPFrail (Lavan et al, 2017). OncPal has only been clinically validated at one centre by the tool authors, and STOPPFrail has not been clinically validated. No studies have directly compared these tools.

Aims. To assess the efficacy (positive predictive value, PPV) and safety (negative predictive value, NPV) of OncPal and STOPPFrail in comparison to a clinical review for palliative patients.

Methods. The electronic records of hospitalised patients referred to palliative care were retrospectively reviewed. Two geriatricians and a clinical pharmacologist each independently reviewed the medicines on admission and a clinical consensus to stop or continue each medicine was reached by simple majority. The clinical consensus was considered the gold standard assessment. The fourth author separately applied both deprescribing tools to the medicines on admission of each patient.

Results. 30 patients with a median (range) of 11 (6-23) medicines on admission were included in the study. The median (range) age was 73 (43-91) years, cancer was the primary diagnosis for 20 patients, and only two patients had a prognosis of greater than one year. The clinical consensus was to cease 75/360 (21%) medicines on admission. OncPal and STOPPFrail had PPVs of 65% and 60%, and NPVs of 89% and 86%, respectively. The medicines deprescribed by both tools, but not the clinical experts, were considered unlikely to cause significant clinical harm.

Discussion. Both tools performed similarly. The significant false positive rate for both tools may limit clinical utility, although posed a low safety risk in this population. There is scope to refine both tools.

Lavan et al (2017) Age Ageing 46(4):600-607
Lindsay et al (2015) Support Care Cancer. 23(1):71-8

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Monitoring for valproate toxicity in patients with hypoalbuminemia

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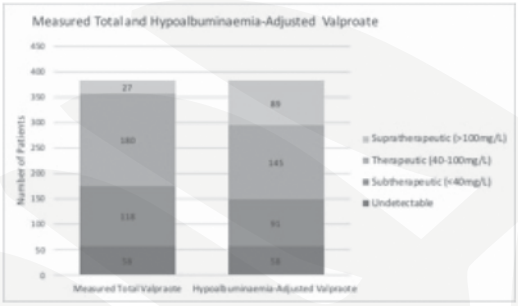
Introduction. Valproate is highly protein-bound (>90%) with saturable protein binding and non-linear pharmacokinetics. The unbound component is responsible for antiepileptic activity and adverse effects. The upper limit of the reference range reflects the likelihood of increased toxicity with increasing concentration, but the implications of the increased free component with low albumin may not be recognised when only total concentrations are monitored. The Hermida equation was developed and validated to account for hypoalbuminaemia, however correlation with patient outcomes or toxicity remains unknown.



Aims. To assess if hypoalbuminaemia is associated with increased adverse drug reactions/ toxicity or delayed recognition of toxicity in patients with epilepsy undergoing drug monitoring using total valproate levels.

Methods. Single-centre retrospective cohort study of patients diagnosed with epilepsy undergoing TDM for sodium valproate between January 2018 and December 2018.

Results. Valproate concentrations were monitored for 383 patients with a total of 755 tests performed between January-December 2018. Moderate-severe hypoalbuminemia (≤30 g/L) was noted in 48 (12.5%) patients and 81 (21.1%) patients were aged >65 years. Measured total valproate trough concentrations were undetectable for 58 (15.1%) patients. Supratherapeutic (>100mg/L) total concentrations were recorded for 27 patients (7.0%), which increased to 89 (23.2%) when adjusted for albumin (p<0.001). Medication related neurological adverse effects were documented in 113 patients (29.5%), with many of them having had no documented seizures for over 10 years.

Discussion. The value of therapeutic drug monitoring is in ensuring appropriate interpretation of measured levels to influence prescribing and patient care. While measuring free drug levels in hypoalbuminemia would be ideal, the adjustment equation helps highlight patients who may need further assessment of potential drug-related toxicity.

Patsalos P et al (2018). Ther Drug Monit. 40(5):526-548.
Hermida J et al (2005). J Pharmacol Sci. 97(4):489-93.
Murray L et al (2015). Toxicology Handbook 3rd Edition. Chatswood, Elsevier Australia

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Medication Adherence in Gout: Identifying high-risk patient groups in an Australian clinical setting.

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Introduction. Gout is the most common inflammatory arthritis in men, affecting 1.6% of Australians in primary care. In the short-term, gout causes acute episodes of debilitating pain. However, in the long-term, it can result in joint damage, disfiguring tophi, and renal impairment. In gout, urate is deposited in the joints causing inflammation and pain. Urate-lowering therapy (ULT) is effective in lowering serum urate, with adherence to ULT being paramount for improving outcomes in gout. Despite this, adherence to ULT remains below 50%, and is lower compared to other chronic diseases. To date, no quantitative studies have examined ULT adherence and its associations in Australia.

Aims. We aimed to determine proportions of study participants taking ULT and adherent to ULT; examine factors associated with ULT-taking and adherence behaviours in this population; and examine associations of these behaviours with patient outcomes, particularly serum urate.

Methods. Baseline data from a randomised controlled trial of 309 participants, who have had one or more gout attacks in the past 12 months, have access to a smartphone or tablet device and the Internet, and reside in Australia. Patient-reported survey data were used to determine ULT-taking and adherence behaviours, and serum urate data were obtained from participants' general practitioners. Descriptive statistics were used to describe patient characteristics, taking ULT, adherence, and outcomes. Multivariable logistic regression was used to obtain odds ratios.

Results. 66.3% (95% CI: 60.9% - 71.4%) of participants reported taking ULT, and 52.7% (95% CI: 40.6% -54.1%) of those taking ULT reported being adherent to ULT. Patients at risk of not adhering to ULT or not taking ULT were younger, male or employed, or lived alone, engaged in binge drinking or smoking, had fewer comorbidities or did not see a specialist in the past 6 months. In the multivariable model, only older age (OR: 1.03; 95%CI: 1.01-1.05) and having seen a specialist (OR: 1.43; 95%CI: 1.06-1.92) remained significantly associated with ULT adherence. Participants adhering to ULT were more likely to achieve target serum urate of 0.36 mmol/L (adjusted OR: 3.51; 95% CI: 2.02-6.09).

Discussion. These results are consistent with findings in the international literature. Patients may not be receiving adequate care and support to take ULT or be adherent to ULT if they are not seeing a specialist for management of gout. Younger patients may need more self-management support to improve ULT adherence.

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Activity and potential biomarkers of evofosfamide in patient-derived and cell line xenograft models of head and neck cancer

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Introduction. Evofosfamide (TH-302) is a clinical-stage hypoxia-activated prodrug designed to selectively target a potent DNA crosslinking nitrogen mustard to the poorly oxygenated (hypoxic) regions of tumours and spare normal tissue. Since hypoxia is a known marker of poor prognosis and chemoradiotherapy failure in head and neck squamous cell carcinoma (HNSCC), evofosfamide is a potential therapeutic strategy in this setting that would ideally be co-developed with appropriate biomarker support to identify the patients most likely to benefit from therapy.

Aims. To evaluate the anticancer activity of evofosfamide in HNSCC patient-derived and cell line xenograft tumour models and perform an initial investigation of biomarkers that might predict sensitivity to evofosfamide in HNSCC. Methods. The anticancer efficacy of evofosfamide was assessed in 13 novel patient-derived (PDX) and 4 cell line xenograft (CLX) models of HNSCC. Tumour histology and hypoxia status were determined by haematoxylin/eosin and pimonidazole staining. PDX models were genetically characterised by NanoString, CLX models by RNA sequencing.

Results. The PDX tumours displayed more intratumour heterogeneity than the CLX tumours and closely resembled the histology and hypoxia gene signature expression of the patient tumours they were derived from. Evofosfamide (50 mg/kg qdx5 for 3 weeks) significantly inhibited tumour growth in several HNSCC xenograft models. Antitumour efficacy did not strongly correlate with tumour hypoxia, determined either by pimonidazole staining or by expression of the Toustrup hypoxia gene signature, nor with candidate evofosfamide sensitivity genes: *MKI67*, *POR* and *SLFN11*.

Discussion. These data confirm that evofosfamide has antitumour activity in clinically-relevant tumour models of HNSCC and support further clinical evaluation of this drug in HNSCC patients. Further research is ongoing to identify those factors that alongside hypoxia can influence sensitivity to evofosfamide that could act as predictive biomarkers to enable precision medicine therapy of evofosfamide in HNSCC.

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Effect of CYP2C19 phenotype and physiological differences on the pharmacokinetics of clopidogrel and its metabolites in European and Japanese populations.

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Introduction. Clopidogrel is extensively metabolised by 1) esterases (CES1; 85%) to a carboxylic acid which is glucuronidated (UGT2B7) to the inactive acyl glucuronide (potent CYP2C8 inhibitor), and 2) CYP450s (2C19, 1A2, 2B6; 15%) to 2-oxo-clopidogrel which is metabolised (3A4, 2C19, 2B6, 2C9) to the active metabolite H4. CYP2C19 activity is strongly associated with clopidogrel treatment response. CYP2C19 extensive metabolisers (EM) are more common in the European populations, while poor metabolisers (PM) are more common in East Asian populations.

Aims. To investigate the effect of CYP2C19 activity on the pharmacokinetics of clopidogrel (loading dose 300 mg, daily dose 75 mg) and its metabolites in populations of European and Japanese ancestry using a model-based approach.

Methods. Minimal physiologically-based pharmacokinetic (PBPK) models (parent and metabolites) were built for Japanese and European populations using the SimCYP Simulator (V18). PK data by CYP2C19 phenotype in European (IV, oral doses) and Japanese (oral doses) subjects from 5 studies were used to build and verify these models (acceptance criteria; <2-fold difference observed to predicted PK parameters). To verify the contribution of the CYP2C19 pathway in both ethnic groups, the effect of fluvoxamine (50 mg) on clopidogrel and metabolite exposures was investigated by CYP2C19 phenotype (10 trials, 10 individuals).

Results. Mean predicted systemic CL of clopidogrel was 90 L/h in European and Japanese populations. Assuming the same CYP2C19 abundances by phenotype, PK predictions in Japanese and European populations were well described with similar predicted and observed H4 AUC ratios (CYP2C19 PM/EM; European 0.35; Japanese 0.40). CYP2C19 activity had a minor effect on acyl glucuronide formation but an important effect on H4 metabolite formation (efficacy). The major driver of the population difference in H4 metabolite PK is the frequency of CYP2C19 polymorphisms.

Discussion. PBPK models for clopidogrel and metabolites were developed and verified for populations of European and Japanese ancestry. CYP2C19 activity has an important effect on H4 formation but a minimal effect on acyl glucuronide formation. The risk of interactions with CYP2C8 substrates is similar in both ethnic groups.

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ADME genes predict cancer patient survival: a comprehensive analysis of 21 different human cancers

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Introduction. 298 genes are classified by the PharmaADME Consortium as ADME genes that are involved in drug Absorption, Distribution, Metabolism, and Excretion (ADME). ADME genes also have critical roles in detoxifying and clearing numerous carcinogens and cancer-growth modulating molecules. Therefore, the expression of ADME genes in cancer cells impacts cancer treatment efficacy and cancer progression. However, a comprehensive assessment of ADME gene expression in cancers and their association with patient survival is lacking.

Aims. To define the ADME gene expression profiles in various cancers and their association with patient survival.

Methods. RNAseq data and clinical data of 7983 patients from 21 different types of cancers (BLCA, BRCA, CESC, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, READ, SARC, SKCM, STAD, UCEC) were obtained from The Cancer Genome Atlas (TCGA) project. The correlation of ADME gene expression levels with cancer patient survival was assessed using Multivariate Cox-regression analysis and Kaplan-Meier analysis.

Results. ADME genes were differentially expressed with a unique complement ranging from 181 to 248 genes in different cancers. Overall, the mRNA levels of 252 ADME genes showed significant correlation to patient survival in at least one cancer. 19 ADME genes (*ABCB7*, *ADH1B*, *ADH5*, *ALDH2*, *ALDH4A1*, *AOX1*, *CYP27A1*, *EPHX1*, *EPHX2*, *FMO3*, *HAGH*, *KCNJ11*, *MGST2*, *PDE3A*, *SLC16A1*, *SLC22A5*, *SLC7A5*, *SULT2B1*, *TAP1*) were significantly associated with patient survival in 7-9 types of cancers (>30%), whereas 16 other ADME genes (*ABCC9*, *ABCG1*, *ADHFE1*, *ALDH3A1*, *ALDH7A1*, *CHST1*, *CYP19A1*, *CYP2D6*, *CYP2E1*, *DHRS12*, *FMO2*, *FMO4*, *HSD11B1*, *NNMT*, *SLC29A1*, *SULF1*) were significantly associated with patient survival in 6 types of cancers (>25%). Of these 35 ADME genes, nine genes showed either positive (*ABCC9*, *CYP19A1*, *SLC16A1*, *SLC7A5*, *SULT2B1*) or negative (*DHRS12*, *FMO4*, *HAGH*, *MGST2*) correlations consistently across cancers, suggesting their similar role in survival in all cancers, whereas the other 26 genes showed correlation positively in some cancers but negatively in others, suggesting their cancer-specific impact on survival.

Discussion. This study represents the first comprehensive assessment of ADME gene expression profiles in cancers and their correlation with patient survival; which likely relates to their roles in detoxifying/clearing drugs and cancer-growth modulating molecules. Our results indicate that many ADME genes predict cancer patient survival; further analysis of specific mechanisms involved could provide new opportunities for biomarker or therapy development.

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A model to inform in-vivo lignocaine pharmacokinetics from a controlled release device

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Introduction. Data were available for a controlled release device for lignocaine tested in 8 sheep. Interpretation of these data in context of previous in-vitro work and for planning future clinical studies was required.

Aims. To describe pharmacokinetics of lignocaine following device placement in sheep, and to confirm whether release was similar to that in-vitro.

Methods. Lignocaine concentrations from the peritoneal and plasma were taken 1, 2, 4, 6, 24, 48, 72, 120 and 168 h after device placement. Cumulative lignocaine release data from a prototype device, obtained under sink in-vitro conditions, were used to estimate a release rate constant (K_{rel}) and bioavailability (F). Drug was assumed to enter the peritoneal and absorb into the plasma (first order absorption, T_{abs}), or to divert directly to first pass metabolism. All other elimination was assumed to be from the plasma. Data were analysed with NONMEM (NONMEM 7.4, Globomax LLC, Hanover, MD, USA).

Results. K_{rel} (0.025 h^{-1}) and F (0.95) were fixed at in-vitro estimates, with 9.4% (95%CI 1.2-19.2%) estimated as instant release from the device and Tabs 0.067 h (0.002-0.212 h). A lack of iv data made plasma parameters difficult to estimate so these were fixed at literature values. The final model predicted similar drug release to that obtained in-vitro and in-vivo (model 80.8% at 72 h, 93.7% at 168 h, versus 84.2% and 98% in sheep Figure 1).

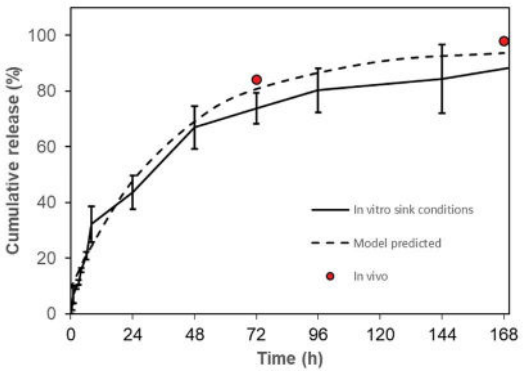

Figure 1 Cumulative release of lidocaine: in-vitro (solid line), predicted release in-vivo from the model (broken line). Average lidocaine in the device at day 3 (n=3) and 7 (n=5) in sheep (circles).

Discussion. In-vitro data was used to inform pharmacokinetic modelling of in-vivo drug release. Information gained here will support development of a patient-ready controlled release system to achieve desired lignocaine delivery.

We acknowledge the following for various contributions to generating the data used in this analysis, and the wider research group: Andrew Hill[3], Manisha Sharma[3], Priyanka Agarwal[3], Wiremu MacFater[3], Ahmed Barazanchi[3], Kaushik Chandramouli[3], Prabhat Bhusal[4]: University of Auckland[3], Auckland, NZ; University of Otago[4], Dunedin, NZ.

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Assessment of inter-ethnic differences in imatinib dosing regimen using physiologically based pharmacokinetic (PBPK) modelling and simulation

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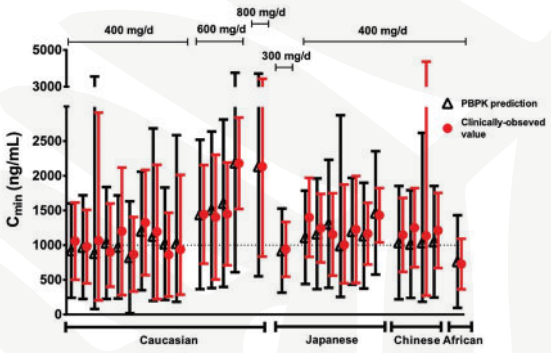
Introduction. There is a potential for ethnic differences in imatinib systemic exposure based on differences in body size and expression of drug metabolizing enzymes / proteins involved in imatinib disposition.

Aims. This study implemented a PBPK approach to investigate inter-ethnic differences in imatinib PK and dosing regimens.

Methods. A PBPK model of imatinib was built (Simcyp Simulator version 17) and verified using published PK data from different ethnic groups. Predictive utility of this model for imatinib trough concentrations (C_{min}) was evaluated across different dosing regimens and ethnic groups. The impact of ethnicity on imatinib dose was then assessed based on the established C_{min} targets.



Results. The PBPK model described imatinib PK in patients from 4 ethnic groups and demonstrated a good predictive performance in simulating the attained C_{min} following different dosing regimens, with prediction-fold differences within 1.25-fold of the clinically-reported values (Fig). PBPK simulation suggested a similar dose of imatinib (400 – 600 mg/d) to achieve the targeted C_{min} (1,000 – 3,200 ng/mL) in Caucasian, Chinese and Japanese patients. The PBPK simulation indicated that the African population may benefit from a higher initial dose of imatinib (600 – 800 mg/d), due to a higher CL/F of imatinib compared to other ethnic groups, however the clinical results available were limited.

Discussion. A PBPK model for imatinib was successfully developed with a capability to predict PK of imatinib in different populations. PBPK simulations highlighted a potential ethnic difference in the recommended initial dose of imatinib, particularly between the European and African populations, but not for Chinese and Japanese patients.

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Molecular basis of the reduced enzyme activity associated with the cytochrome P450 2C9*3 (Ile359Leu) variant

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Introduction. Cytochrome P450 2C9 (CYP2C9) is an enzyme of major importance in drug metabolism [1]. In particular, CYP2C9 is responsible for the metabolic clearance of many drugs that have a narrow therapeutic index (e.g. phenytoin, S-warfarin, sulfonylurea hypoglycaemic agents). CYP2C9 is highly polymorphic with over 60 known alleles. Of these, the Ile359Leu (CYP2C9 *3) variant is associated with > 80% reduction in the catalytic activity of nearly all substrates metabolised by this enzyme. Position 359 is not located within the substrate binding domain, and the molecular basis of the reduced enzyme activity remains unknown despite intense investigation over many years.

Aim. To elucidate the molecular basis of the reduced enzyme activity of CYP2C9*3.

Methods. Molecular Dynamics Simulations (MDS) [2] were used to model the plasticity of the CYP2C9*3 and wild-type CYP2C9 proteins. Simulations were performed in the absence and presence of prototypic substrates, using the GROMACS simulation package in conjunction with the GROMOS53A6 force field.

Results. MD simulations of the CYP2C9*3 structure show that Leu359 restricts the conformational freedom of the side-chain of Tyr308, which is located in the I-helix. The restricted conformation of Tyr308 results in a favourable CH-π interaction with Pro471, adjacent to the β4-1 region. The altered conformation of Tyr308 and Pro471 leads to significant displacement of the loop positioned between the K-helix and the β1-4/β4-1 region, thereby constricting the ligand binding domain above the heme moiety. As a result, catalytic efficiency is predicted to decrease. By contrast, the wild-type CYP2C9 active site is more plastic and open, with improved ligand accessibility.

Conclusion. The reduced activity of CYP2C9*3 arises from 'knock-on' effects that occur within the enzyme active site as a result of substitution of isoleucine with leucine.

1. Miners JO, Birkett DJ. (1998). Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol., 45, 525-38.
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Discovery of novel peptide ligands for orphan G protein-coupled receptors

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Introduction. G protein-coupled receptors (GPCRs) form the largest family of cell-surface receptors and are involved in nearly all aspects of physiology. However, about 100 human non-olfactory GPCRs have not been definitively paired with endogenous ligands and are considered as orphan receptors. Given their strong potential to influence human physiology and disease processes, these receptors represent a treasure-trove of unexplored biology.

Aim. To identify new peptide ligands from the human proteome that activate orphan GPCRs.

Methods. Capitalising on the wealth of comparative genomics data and leveraging bioinformatic data on human class A GPCRs, we identified common sequence and structural characteristics for known peptide receptors within the orphan receptor clade and we applied statistical and machine learning analyses to identify putative peptide ligands. We then used a multifaceted experimental approach, including 3 complementary pharmacological screening assays to capture orphan receptor responses. New peptide-orphan GPCR responses were validated in orthogonal G protein and β-arrestin signalling assays, and peptide variants for several pairings were synthesised to identify more potent ligands.

Results. We tested our 218-membered peptide library against 67 orphan and understudied GPCRs and 27 known peptide receptors. We extensively validated our initial peptide hits for receptor specificity and concentration-dependence across 8 different signalling assays. In addition to potential new ligands for nine peptide GPCRs, we discovered and characterised the novel pairings of 17 peptides with five different orphan GPCRs in detail.

Discussion. Our combined computational and pharmacological approach has identified new peptide receptor pairings that expand the human peptidergic signalling network from 348 to 407 interactions (an increase of 17%). We envisage that these findings will lay the platform for many future studies on these peptides and receptors to fully characterise their roles in human physiology and disease.

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Development of Next-Generation Antimicrobial Combination Therapies Against PDR *Klebsiella pneumoniae*

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Introduction. Pandrug-resistant (PDR) *Klebsiella pneumoniae* has been identified by the WHO as one of the 3 top-priority pathogens urgently requiring novel therapeutics. This bacterial 'superbug' causes life-threatening infections, particularly in the critically ill, and polymyxins are often used as the last option. Worryingly, plasmid-mediated polymyxin resistance highlights the urgency to develop novel therapeutics to treat PDR *K. pneumoniae*.

Aims. To employ systems pharmacology and pharmacokinetics/pharmacodynamics (PK/PD) to rationally design and optimise novel polymyxin-based triple antibiotic combination therapy and polymyxin-based phage therapy against life-threatening infections.

Methods. Static time-kill (STK) studies were conducted to examine the PD of polymyxin B (PMB, 2mg/L), rifampicin (RIF, 5mg/L), amikacin (AMI, 20mg/L), meropenem (MER, 50mg/L), and minocycline (MIN, 4mg/L) monotherapy, in addition to double and triple combinations against PDR *K. pneumoniae*. The most promising triple combination, PMB/RIF/AMI, was examined in an *in vitro* one-compartment model (IVM) and a mouse thigh infection model. Population analysis profiles (PAPs) were performed to examine the emergence of polymyxin-resistance. Similar approach was also utilised to rationally design and optimise novel phage/PMB combination.

Results. In the STK studies, all monotherapies were ineffective against all six *K. pneumoniae* isolates. PMB-based triple combinations showed synergistic bacterial killing at 1 and 4h, followed by regrowth at 24h. Based on the results, PMB/RIF/AMI was the most effective triple combination. The IVM results demonstrated sustained synergistic bactericidal activity of PMB/RIF/AMI over 48h, reducing up to 5 $\log_{10}$CFU/mL. In mice, PMB/RIF/AMI displayed synergistic bacterial killing by reducing 2-3 $\log_{10}$CFU/thigh at 24h. PMB/phage combination was able to eradicate PDR *K. pneumoniae* both in STK and mouse bacteraemia infection model.

Discussion. This study demonstrated that PMB/RIF/AMI and PMB/phage are effective against PDR *K. pneumoniae* and results in minimal bacterial regrowth and warrant future clinical studies.

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Translational toxicovigilance: preventing drug related harms through legislation and policy

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Introduction. In 2018 poisoning accounted for 7% of premature years of life lost in Australia (more than the road toll), and there has been a 50% increase in poisoning deaths in the past decade (Australian Bureau of Statistics, 2019). Analgesics (including opioids, simple analgesics and gabapentinoids) are commonly involved in poisonings and misuse. Up-scheduling is a key intervention used to target pharmaceutical misuse and harms. Codeine was up-scheduled to Schedule 4 in 2018, in a controversial decision. Modified release (MR) paracetamol is particularly dangerous in overdose. It has been banned in Europe and in Australia it will be up-scheduled to Schedule 3 in 2020. Pregabalin has an abuse potential, and the Therapeutic Goods Administration (TGA) is currently considering up-scheduling.

Aims. To estimate trends in poisonings with codeine, paracetamol, and pregabalin. To evaluate the effect of codeine up-scheduling on poisoning calls.

Methods. A retrospective analysis of calls to the New South Wales Poisons Information Centre (NSWPIC, Australia's largest PIC, taking 50% of Australia's poisoning calls).

Results. We observed a large, significant drop in codeine overdoses following 2018 up-scheduling (-50.8%, 95%CI -79.0 to -22.6, P<0.001). There was no increase in poisonings with higher strength codeine products (30mg per dose unit). Paracetamol is the most common intentional poisoning call handled by NSWPIC, (22,997 cases, increasing by 77% overall or 3.8% per year, 2014-2017, P<0.001). Paracetamol overdose size has increased significantly in this time (P<0.001). MR paracetamol ingestions increased by 38.3% per year, 2004-2017. Intentional poisonings involving pregabalin increased by 53.8% per year, 2005-2016, P<0.001.

Discussion. Codeine up-scheduling successfully reduced harms from poisonings. We document increasing harm from other analgesics, namely MR paracetamol and pregabalin. Poisoning data will be key in evaluating any future scheduling changes with these medicines.

Australian Bureau of Statistics (2019), *Causes of Death, Australia, 2018,* cat. no. 3303.0, viewed 1 October 2019 https://www.abs.gov.au/ausstats/abs@.nsf/mf/3303.0

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Gain from pain: using venomous animals to explore new nociceptive pathways

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Introduction. Animal venoms are complex mixtures that typically contain hundreds of peptide and protein toxins. A primary role of venom for many animals is predation, where specific toxins act to subjugate prey by targeting vital processes in one or all of the nervous, musculoskeletal or cardiovascular systems. But almost all venomous animals *also* use their venoms for defensive purposes—many solely. Defensive envenomations are often associated with intense pain and my hypothesis is that this pain is produced by toxins that directly target sensory neurons, hijacking or overstimulating neuronal transmission.

Aims. The goal of my research is to identify, from a range of pain-producing animal venoms, the responsible pain-causing (algogenic) toxins and to determine their mechanism of action.

Methods. Venom samples were acquired from numerous species with characteristically painful stings and the composition of several venoms was determined using a combination of venom proteomics and venom-gland transcriptomics. High-content calcium imaging of mammalian sensory neurons was used to guide the isolation of algogenic toxins. Calcium imaging and electrophysiology were used to determine cellular and molecular mechanisms of action.

Results. We have identified new algogenic toxins from a range of venoms. Different venomous animal lineages employ distinct structural classes of algogenic toxins. A common convergent mechanism of action is the targeting of cell membranes to generate a leak in ion conductance. In excitable cells, such as mammalian sensory neurons, this leak in ion conductance shifts the membrane potential to threshold, initiating neuronal depolarisation, an action, which on nociceptors, results in immediate pain. Other more specific mechanisms also exist, including the activation (or delayed inactivation) of specific ion channels and receptors involved in normal sensory reception and transduction.

Discussion. The identification and characterisation of new algogenic toxins has provided new knowledge about methods of chemical defence by venomous animals and has the potential to elucidate new components of mammalian pain signalling pathways. A better understanding of our own pain physiology may ultimately lead to the development of new or improved pain treatments.

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Comparison of two mice models of non-alcoholic steatohepatitis

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Introduction. Non-alcoholic steatohepatitis (NASH) is characterised by obesity, liver steatosis, inflammation, hepatocyte ballooning, and fibrosis. Although there is clear unmet clinical need, current animal models, including the methionine choline deficient (MCD) diet induced model, lack some of the key features of human NASH.

Aims. We hypothesised that the streptozotocin (STZ) and high fat diet (HFD) mouse model of NASH recapitulates human NASH features, and maybe a more appropriate model than the MCD diet induced NASH for drug efficacy evaluation.

Methods. NASH was induced in C57BL/6 male mice (6wks old) using a combination of STZ (3x55mg/kg/per day, i.p.) and high fat diet (42% fat) for 20 weeks. The corresponding sham mice received citric acid vehicle and normal diet. This model is compared to the published MCD diet model in which the C57BL/6 male mice (8wks old) were fed an MCD diet for 4 weeks. Plasma and liver samples were collected at study end and used to quantify markers of liver injury. NASH features such as steatosis, inflammation and ballooning were scored using H&E staining. Fibrosis score was determined using picrosirius red staining.

Results (mean±SEM)	MCD sham (n=10)	MCD (n=10)	STZ+HFD sham (n=12)	STZ+HFD (n=13)
Body weight (g)	20.6±0.3	19.6±0.2*	33.0±1.1	33.0±0.9
Plasma ALT (U/L)	33.4±3.6	159±9.8*	20.0±2.2	84.5±16*
Plasma AST (U/L)	158±34	315±47*	79.3±11	202±33*
Steatosis (AU)	0.0±0.0	3.0±0.0#	0.1±0.1	2.0±0.3#
Inflammatory (AU)	0.7±0.3	2.3±0.2#	0.3±0.1	0.8±0.3
Ballooning (AU)	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2
Fibrosis (AU)	0.0±0.0	1.3±0.0#	0.6±0.2	1.5±0.3#

*p<0.05 (unpaired t-test) and #p<0.05 (Mann Whitney U test) vs respective sham
AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Results. Overall, both models showed important NASH features. The MCD model showed a more severe phenotype in most features but showed no sign of hepatocyte ballooning (Table). The STZ+HFD model results tended to be milder compared to the MCD model but also showed the key feature of human NASH, namely hepatocyte ballooning.

Discussion. The STZ+HFD-induced NASH may be a more suitable model for evaluating drug efficacy for NASH.

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The role of Transforming Growth Factor β (TGFβ) in the response to Influenza A viral infection.

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Introduction. Asthma and chronic obstructive pulmonary disease (COPD) are characterised by airway fibrosis and worse patient outcomes following Influenza A virus (IAV) infection. While elevated TGFβ levels are implicated in these airway structural changes, the role of this pro-fibrotic cytokine in regulating the immune response to infection is unclear. Previous studies have shown that viral replication is enhanced in TGFβ-treated airway fibroblasts, while lung-specific TGFβ gene deletion affords protection against viral infection in mice (Thomas et al, 2009; Denney et al, 2018).

Aims. To use a lung-specific doxycycline-inducible mouse model of TGFβ overexpression (Lee et al, 2004) to determine whether TGFβ influences IAV infection and subsequent inflammatory and immune responses.

Methods. Four groups of mice (control, TGFβ, IAV, IAV+TGFβ, n=5-13) were randomized to receive water or doxycycline (DOX, to induce TGFβ in club cells) for 8 wks, then saline or IAV ($1x10^2$PFU HKx31 mouse strain) i.n and monitored for 3 days. Fibrosis was assessed in Masson's trichrome sections, and inflammatory and immune responses measured in bronchoalveolar lavage (BAL) & lung samples by cell counts, cytokine ELISAs or bead array, and RT-PCR.

Results. 8 weeks DOX increased fibrosis (subepithelial thickness (μm): control 5.1±1.1; TGFβ 16.5±0.9 μm, p<0.001), with a further 3-fold increase following infection (IAV+TGFβ *cf* TGFβ, p<0.001) despite similar BAL TGFβ levels. Total BAL cells from IAV+TGFβ mice were 60% higher than IAV alone (p<0.01). BAL and/or lung MCP-1, IL-6 and IL-8 followed this pattern. IFNα and interferon-induced genes (IFIT1, IFIT2) elevated by IAV were not attenuated by TGFβ.

Discussion. The unexpected exacerbation of the fibrotic response to TGFβ with IAV may be due other pro-fibrotic factors increased by infection. The amplification of IAV-induced increases in chemokines MCP-1 and IL-8 by TGFβ was consistent with greater inflammatory cell influx. Higher IL-6 levels, also seen with IAV+TGFβ, have been associated with worse response to infection. However, relative viral load still needs to be measured since an impaired immune gene response was not detected with TGFβ. It also remains to be determined if the effects of TGFβ on the response to IAV infection are sensitive to the effects of new therapies targeting its contribution to fibrosis in chronic lung diseases.

Denney (2018) Mucosal Immunol, 11:523-35; Lee (2004) J Exp Med 200:377-389; Thomas (2009) AJRCMB, 41:339-47

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A fresh look at cGMP in innate immune responses

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Introduction. cGMP dampens the innate immune response by decreasing cytokine secretion from lipopolysaccharide (LPS)-induced macrophages (e.g. Ahluwalia et al. 2004). Interleukin 1 associated receptor kinase 3 (IRAK3) plays a pivotal role in suppressing cytokine secretion (Kobayashi et al. 2002). Bioinformatic searches reveal that IRAK3 contains a predicted cryptic guanylate cyclase centre within its pseudokinase domain (Freihat et al. 2014).

Aims. To re-examine roles of cGMP and if mutations in the guanylate cyclase centre in IRAK3 modulate IRAK3 action.

Methods. THP-1 BLUE and HEK293T BLUE cells containing NFκB reporter systems were used. IRAK3 and IRAK3 mutant constructs were transfected into HEK293T BLUE cells. cGMP, IL6 and TNFα were measured using ELISA kits.

Results. cGMP suppresses NFκB activity and TNFα or IL-6 production in THP-1 BLUE cells stimulated by LPS. Recombinant wild type IRAK3 produces cGMP, whereas point mutations in the GC centre and surrounding residues reduce cGMP production. HEK293T BLUE cells transfected with wild type IRAK3 show reduced NFκB after LPS stimulation while those transfected with IRAK3 mutants with reduced cGMP-generating capacity failed to suppress LPS-induced NFκB activity. Sub-nanomolar cGMP rescues the suppressive effect of IRAK3 only if mutants are present.

Discussion. Low levels of cGMP clearly have role in modulating immune cell responses. The cryptic enzymatic action of IRAK3 in generating cGMP may be involved in the regulatory function of IRAK3 where the presence of cGMP may selectively affect downstream signalling pathway(s) by modulating binding and/or activity of nearby interacting proteins involved in the inflammatory cascade.

Ahluwalia A et al. (2004) Proc Natl Acad Sci USA 101:1386–1391
Kobayashi K et al. (2002) Cell 110:191-202
Freihat L et al. (2014) Biochem Soc Trans 42:1773-1779

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Effects of prolonged exposure to paracetamol on late gestation rat placenta

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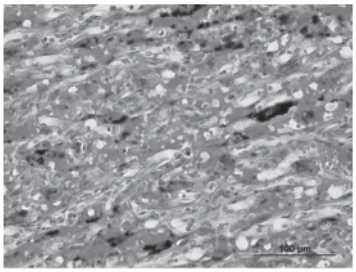

Introduction. Paracetamol (acetaminophen) is one of the most commonly used drugs and over 70% of women take it during pregnancy (Werler et al., 2005). However, its ability to cross the placenta and potential toxicity in placenta and fetuses is unknown.

Aims. 1. To measure placental transfer of paracetamol in pregnant rats repeatedly exposed to two clinically relevant doses of paracetamol, 2. To determine any morphological and functional changes in the placenta after prolonged drug exposure in the dam

Methods. Pregnant Sprague Dawley rats at E15 were injected IP with either 3.75mg/kg (low dose) or 15mg/kg (high dose) paracetamol twice daily over 4 days. At E19 a radioactive tracer ([^{3}H]acetaminophen or [^{14}C]sucrose) was injected iv. (femoral vein) of terminally anaesthetised (urethane) dams and samples of time matched fetal and maternal blood collected to quantify maternal to fetal transfer of the tracers using liquid scintillation counting (Kohen et al, 2019). Placentae from these animals were fixed for histology. In another group of similarly treated dams, individual fetuses were injected IP with [^{14}C]sucrose and blood samples collected after 30 minutes to quantify fetal to maternal transfer of sucrose. Gene and morphological changes in the placentae were determined using RNA-sequencing and immunohistochemistry respectively.

Results. Multiple injections of a high clinical dose of paracetamol into the dam resulted in (i) increased transfer of sucrose from fetus to the mother, double that of low dose treated dams, (ii) increased expression of many inflammatory markers including cytokine Il1ß in the placental tissue, (iii) increased deposits of hemosiderin (illustrated above, arrow) in placental macrophages and prominent structural damage with increased accumulation of maternal blood.

Discussion. Prolonged exposure of pregnant rats to a high clinical dose of paracetamol can induce placental inflammation and result in altered function of the organ as demonstrated by increased transfer of fetal-derived substances. This could have clinical relevance in several tests routinely used in human pregnancy.

Supported by CASS Foundation. Werler et al (2005) Am J Obstet Gynecol 193:771-777; Koehn et al (2019) F1000Res 8:1372

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Mediators from silica-treated macrophages influence contraction of collagen gels seeded with human lung fibroblasts.

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Introduction. Silicosis is caused by exposure to inhaled crystalline silica, and has recently emerged as an epidemic of occupational lung disease in Australia, attributed to the unsafe dry cutting of stone benchtops. This disease is initiated by phagocytosis of silica particles by alveolar macrophages (AMs), which then release inflammatory and pro-fibrotic cytokines such as IL-1β and TGF-β, initiating tissue damage and progressive fibrosis. *In vitro* assays to study these processes are required for assessment of novel therapies for this incurable disease.

Aims. To establish assays to measure the effects of silica on AMs, and of the mediators released by these cells on lung fibroblasts, using contraction of collagen gels seeded with fibroblasts by as an indirect measure of fibrosis.

Methods. THP-1 monocytes were differentiated to AMs, before treatment with silica (1, 10, 100µg/mL, Sigma) for up to 72 hr to induce cytokine release. After treatment, Cell-Titer Glo 2.0 viability assays were performed, and silica particles in fixed AMs were counted using polarizing microscopy. Collagen gel contraction assays were performed in gels seeded with primary human lung fibroblasts. Decreases in gel areas were measured over 72 hr, anticipating increased cell-mediated contraction by treatment with conditioned media (CM) from silica-treated AMs.

Results. Silica did not reduce AM viability. Ingested articles were only evident in AMs treated with 100µg/mL silica (29 ± 4% cells containing silica, 1-18 particles/cell, n=3 independent experiments in duplicate). Fibroblast gel contraction was cell-density dependent. At an intermediate density (1.25 x 10^6 cells/ml), gel areas decreased by 41 ± 5% at 72 hr (n=6 primary cultures). Matched gels from the same cultures contracted ~40% less in the presence of CM.

Discussion. Inhibition of lung fibroblast-mediated gel contraction by CM from silica-treated AMs was unexpected, but may be due to the balance of pro- and anti-fibrotic mediators released by AMs in this experimental setting. Further studies are required to define the specific cytokines present, and assess the direct effects of this CM from silica-treated AMs compared to its individual components on collagen synthesis by human lung fibroblasts, with a view to test potential interventions to limit silica-induced fibrosis.

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In vivo LPS exposure differentially alters intrapulmonary artery reactivity in mouse precision cut lung slices

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Introduction. Acute respiratory distress syndrome (ARDS) results in a decreased quality of life, including increased risk of pulmonary hypertension. In animal models, ARDS is often induced by lipopolysaccharide (LPS) exposure which disrupts the pulmonary epithelial and endothelial barrier and increases the release of pro-inflammatory cytokines. With the application of the precision cut lung slice (PCLS) technique, the effect of *in vivo* LPS on reactivity of small, intrapulmonary arteries to disease-relevant constrictors and clinically-relevant dilators can be assessed .

Aims. To assess whether vascular dysfunction of intrapulmonary arteries can be induced via *in vivo* LPS exposure.

Methods. 8-week old male C57BL6 mice were anaesthetized with isoflurane before administration of LPS (10mg/50ml, intranasal) or 50ml saline daily for 4 days. Mice were culled the following day (0.3ml pentobarbital sodium, 325mg/ml i.p.) for preparation of PCLS or for collection of bronchoalveolar lavage fluid (BALF) and lungs. Changes in area of arteries (80-200µm diameter) to constrictors U46619, endothelin-1 (ET-1) or 5HT, or to PDE5 inhibitor sildenafil (SIL) or NO donor S-nitroso-N-acetylpenicillamine (SNAP) were assessed *in situ* under phase contrast microscopy.

Results. LPS *in vivo* exposure caused a 5-fold increase in cell infiltration in BALF (cellsx10^5/ml: saline 1.6±0.8 n=16; LPS 8.8±1.3 n=17; $p<0.05$) and a 125-fold increase in neutrophils (saline 0.04±0.01 n=7; LPS 5.0±0.9 n=9; $p<0.05$). Furthermore, BALF protein levels increased almost 2-fold (mg/ml: saline 0.20±0.03 n=16; LPS 0.38±0.04 n=18; $p<0.05$). Reactivity was also altered whereby LPS exposure increased artery contraction to U46619 (maximum % reduction in lumen area: saline 19±3% n=7; LPS 43±8% n=6; $p<0.05$). A trend to increased vasoconstriction to ET-1 was also observed (max: saline 27±4% n=7; LPS 46±11 n=7), but reactivity to 5HT was unchanged. Vasodilation to SIL was selectively impaired by LPS exposure (maximum % relaxation: saline 89±7% n=4; LPS 48±6% n=4; $p<0.05$), however, responses to SNAP were unaffected.

Discussion. The inflammatory response to LPS, characterised by neutrophilia, induces differential effects on artery contraction and vasodilation in PCLS. LPS may selectively increase contractile signalling to different agonists. The loss of relaxation to sildenafil, but not SNAP, suggests that exogenous NO may be required to overcome impaired basal cGMP accumulation to reduce increased vascular contraction in inflamed arteries.

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Identification of CXCL17 as an endogenous inhibitor of CXCR4

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Introduction. CXCL17 is the most recently described chemokine ligand and is highly expressed in mucosal tissues. CXCL17 is associated with innate responses, acts as a chemoattractant for monocytes and macrophages, and plays a role in tumour development [1]. However, CXCL17 is poorly characterised and initial reports indicating that CXCL17 is an agonist for the GPCR GPR35 [2] have not been replicated [3].

Aims. As the chemokine receptor CXCR4 is highly expressed in many immune cells that respond to CXCL17, our aim was to investigate if CXCL17 modulates CXCR4 signalling and ligand binding.

Methods. NanoBRET completion ligand binding was performed in live HEK293 cells or membrane preparations expressing Nluc/CXCR4. HEK293 cells transiently transfected with CXCR4/Rluc8 and β-arrestin2/Venus or $G\alpha_{i1}$/Nluc and Venus/$G\gamma_2$ were used to investigate CXCR4-mediated β-arrestin2 recruitment or G protein activation respectively. BRET was measured on a PHERAstar plate reader (BMG) following substrate addition.

Results. In NanoBRET competition ligand binding studies, CXCL17 inhibited CXCL12-AF647 (12.5 nM) binding to Nluc/CXCR4 in cells (pKi; 6.33 ± 0.33, n=4) but not membranes (n=4). In β-arrestin2 recruitment experiments, CXCL17 resulted in a decrease in constitutive BRET between CXCR4/Rluc8 and β-arrestin2/Venus (pIC_{50}; 7.24 ± 0.11, n=3) and inhibited the increase in BRET mediated by exogenous CXCL12 (pIC_{50}; 6.97 ± 0.21, n=6). Similarly CXCL17 inhibited CXCL12 mediated G protein activation (pIC_{50}; 6.73 ± 0.62, n=4) as well as reducing constitutive CXCR4 mediated G protein activation. CXCL17 did not modulate G protein activation mediated by other chemokine receptors (n=4).

Discussion. This work shows for the first time that CXCL17 is a selective low affinity inhibitor of CXCR4 ligand binding and signalling. Moreover these data suggest that CXCL17 inhibits CXCR4 by a unique mechanism of action that requires an intact cellular environment.

[1] Ohlsson et al., (2016) Br J Cancer. 114(6): 697-–703, [2] Maravillas et al., (2015) J Immunol 194: 29-33, [3] Binti et al., (2018) J Immunol 201:714-724.

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One inhibitor, two mechanisms ? evidence for allosteric regulation of the lipid kinase PI3Ka

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Introduction. The class IA phosphatidylinositol-3 kinase PI3Kα, phosphorylates the membrane embedded phospholipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) in response to growth factor activation of tyrosine kinase linked receptors. To perform the reaction PI3Kα needs to bind ATP, recognise activated growth factor receptors and interface with the cells plasma membrane. The enzyme is also activated by mutation in many cancers, and this has driven the discovery, development and clinical application of inhibitors that block the ATP substrate binding site, yet the effect of drug binding on facets of enzyme activity other than catalysis is unknown.

Aims. To investigate the effects of drug binding on the PI3Kα membrane interaction.

Methods. We used a Förster resonance energy transfer (FRET) system along with biolayer interferometry and bespoke chemical tools, to investigate the effect of lipid kinase inhibitors on PI3Kα binding to mixed lipid liposomes.

Results. We identified one PI3K inhibitor, omipalisib that had a dramatic effect on wild type PI3Kα membrane binding. In dissecting the contribution of different drug-ATP binding site interactions to the effect on membrane binding, we developed new molecules that led to the unexpected identification of a drug binding site outside of the active site. The effect of omipalisib was only observed in the PI3Kα enzyme when the enzyme mimicked a growth factor receptor activated state.

Discussion. These data provide evidence that some PI3K inhibitors have two modes of action, they block ATP binding and can regulate the membrane interaction. Further exploration of this could be used to develop new types of PI3Kα inhibitor.

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Investigating the role of the calcium-sensing receptor in airway contraction using mouse precision cut lung slices

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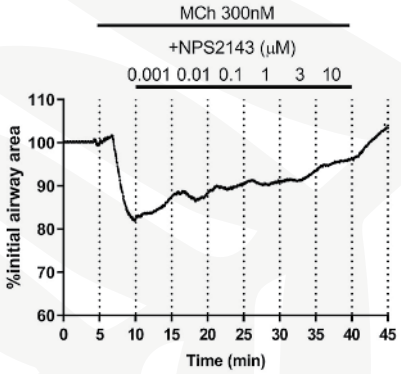
Introduction. The CaSR detects changes in extracellular calcium to maintain homeostasis. This putative drug target is upregulated in asthma, and allosteric agonists for CaSR, such as the polyamines, induce bronchoconstriction. Further, negative allosteric modulators (NAMs; e.g. NPS2143) reduce airway inflammation, remodelling and airway hyperresponsiveness (AHR) in a chronic mouse asthma model. (Yarova et al, 2015). However, whether CaSR NAMs could oppose acute bronchoconstriction is still unknown.

Aim. To investigate the contribution of CaSR to airway reactivity, comparing contraction to CaSR agonist polyamines with methacholine (MCh), and potential dilator effects of CaSR NAMs with salbutamol, currently used in the treatment of asthma.

Methods. Calcium mobilisation assays in CaSR-HEK293 cells were used for quantifying the potency and efficacy of polyamines at the wild type CaSR. Precision cut lung slices (PCLS) from male C57Bl/6 mice were used to visualise changes in airway area to polyamines and MCh, and of salbutamol and CaSR NAMs on pre-contracted airways.



Results. In calcium mobilisation assays, spermine was the most potent (pEC_{50}: 4.51±0.02) compared with other polyamines (agmatine, putrescine, spermidine). Spermine also elicited CaSR-dependent contraction of mouse airways (pEC_{50}: 5.91±0.42; max response: 44.9±6.4% reduction in airway area). NPS2143 reversed 300nM MCh-induced airway contraction (see figure) with higher potency and similar maximum to salbutamol (pEC_{50}: NPS2143 8.25±0.35 salbutamol 5.84±0.34 p<0.01; % relaxation: NPS2143 49.6±9.7% salbutamol 55.4±7.9%, n=5, 4).

Discussion. Spermine elicits airway contraction and negative allosteric modulation of CaSR opposes MCh-induced airway contraction in mouse airways with greater potency than salbutamol. These acute effects identify the CaSR as a novel therapeutic target for the treatment of excessive bronchoconstriction in asthma.

Yarova et al (2015) Sci Transl Med. 7:284

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New strategies to investigate the CC Chemokine Receptor 2 (CCR2) signalling network using phosphoproteomics

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Introduction. Leukocyte recruitment is regulated by chemokines, which are secreted at the site of injury or infection to activate G protein-coupled chemokine receptors on the target leukocytes. CC chemokine receptor 2 (CCR2) is abundantly expressed in monocytes and has long been considered as a target for inflammatory diseases such as atherosclerosis. However, numerous clinical trials of CCR2 antagonists have failed, in many cases due to lack of efficacy in phase II or III. One contributing factor for this failure is the complexity and potential compensatory mechanisms inherent in chemokine signalling networks.

Aims. To gain a deeper understanding of the chemokine-mediated signalling using phosphoproteomics, in order to identify new targets downstream of CCR2.

Methods. We performed a proteomic study using data-independent acquisition (DIA) mass spectrometry to quantify changes in protein and phosphopeptide levels between untreated and CCL2/MCP-1-stimulated TREx 293 cells stably expressing CCR2. Using monocyte-like THP-1 cells and THP-1 cells stably expressing CAMYEL cAMP biosensor (Valkovic et al., 2018), we characterised endogenous CCR2-dependent signalling (calcium mobilisation, cAMP, chemotaxis).

Results. We confirmed the involvement of canonical chemokine-related pathways (MAPK, JAK/STAT and Akt/mTOR) in our phosphoproteomics data and identified, mapped and manually curated additional proteins that have not been associated with CCL2/CCR2 signalling. CCL2 elicited robust CCR2 signalling in THP-1 cells (EC_{50} for cAMP 1.4 nmol/L and calcium mobilisation 38 nmol/L), whereas CCL2-dependent signalling was inhibited by INCB3344 (IC_{50} 17 nmol/L).

Discussion. To the best of our knowledge, this is the first application of DIA to globally quantify the CCL2-CCR2 phosphoproteome. We are currently extending these findings to study CCL2-dependent signalling pathways in physiologically relevant monocyte cell lines and primary cells.

Valkovic AL et al (2018) Pharmacol Res Perspect. 6(5):e00432.

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Low intrinsic G protein efficacy can explain the improved side effect profile of novel opioid ligands

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Introduction. Biased agonism at G protein-coupled receptors (GPCRs) describes the phenomenon whereby some drugs can activate some signalling functions to the relative exclusion of others. Descriptions of biased agonism focusing on the differential engagement of G protein versus arrestins are commonly limited by the small response windows obtained in poorly amplified or less effectively coupled pathways, such as arrestin recruitment. At the mu-opioid receptor (MOR), an unrivalled target for pain treatment, G protein-biased ligands have been proposed to induce less constipatory and respiratory depressant side effects than commonly used opioids. However, it is unclear whether these improved safety profiles are due an arrestin-mediated mechanism or, alternatively, to their low intrinsic efficacy in all signalling pathways.

Aims. We investigated the relationship between *in vitro* efficacy or bias factors with the *in vivo* therapeutic window of opioids, including the most recent and promising MOR biased ligands (oliceridine, PZM21 and SR-17018).

Methods. We quantified efficacy at multiple G protein and arrestin-dependent pathways in model cell lines using Bioluminescence Resonance Energy Transfer (BRET). We then quantified therapeutic indices in terms of the agonist effects on antinociception and respiratory depression in mice.

Results. We show that oliceridine, PZM21 and SR-17018 have low intrinsic efficacy. We also demonstrate a strong correlation between measures of efficacy for receptor activation, G protein coupling and arrestin recruitment for all tested ligands. By measuring the antinociceptive and respiratory depressant effects of these ligands, we show that low intrinsic efficacy of opioid ligands can explain an improved side effect profile in terms of a continuum of existing analgesics.

Discussion. Our results suggest an alternative mechanism underlying the improved therapeutic windows described for novel opioid ligands, which should be taken into account for future descriptions of ligand action at this important therapeutic target.

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Orai channel alterations as a consequence of differentiation in a human neural progenitor cell line

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Introduction: Dysregulation of Orai calcium channels is a feature of numerous pathological conditions, as varied as immune deficiency and cancer. Increasingly, Orai alterations are implicated in neurological conditions, however, evaluation of the potential role of these channels as therapeutic targets is hindered by a lack of understanding of the role of Orai channels in physiological neural processes. ReNcell VM is an immortalised human neural progenitor cell line, which can differentiate from a proliferative stem like state into neural matrices comprised of astrocytes and neurons. This model is a powerful *in vitro* tool for investigating physiological neurogenesis, as well as altered signalling pathways in neurological conditions such as Alzheimer's and Parkinson's disease. To date, however, no work has evaluated Orai channel signalling in ReNcell VM in either of these contexts.

Aims: To assess alterations in Orai isoform expression and functionality in differentiating ReNcell VM cultures.

Methods: ReNcell VM cultures stably expressing the calcium sensor jrCaMP1b were differentiated for up to 14 days and assessed for altered expression of Orai isoforms through RT-qPCR. Functional assays and pharmacological screens were performed with ImageXpress Micro or FLIPR^{TETRA}.

Results: Expression of the neuronal and astrocytic markers, Map2 and GFAP, respectively, increased in differentiating ReNcell VM cultures and coincided with a loss of expression of canonical Orai1 (1.78 fold decrease) and increased expression of Orai2 (2.7 fold increase) and Orai3 (14.5 fold increase). The Orai channel activator STIM1, but not its related isoform STIM2, increased in expression in differentiated neural matrices. Spontaneous calcium oscillations increased with differentiation. Responses to endoplasmic reticulum calcium store releasing agents ATP and carbachol were also altered.

Discussion: This study provides the first evidence of a dramatic remodelling of the calcium signal, and Orai isoform expression as a consequence of differentiation to mature neurons and astrocytes in the human neural progenitor ReNcell VM model.

Amantea D et al. (2018) Front Mol Neurosci. 11:87
Kim D et al. (2015) Nat Protoc. 10(7): 985-1006

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Proteomic analysis of drug metabolising enzymes in extracellular vesicles

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Introduction. Variability in drug exposure as a result of variability in drug absorption, distribution, metabolism and excretion can be accounted for by understanding the enzyme activity and expression. Small extracellular vesicles (sEVs) are released into the bloodstream by organs, containing functional proteins and nucleic acids, and reflect the functional state of that organ. This study aims to quantify activity and expression of CYPs and UGTs in sEVs derived from blood as a source for potential biomarkers.

Methods. For peptide screening, in-gel trypsin digestion was performed. Peptides were separated by liquid chromatography (LC) with a 45 min acetonitrile gradient (BSciexEkspert400nanoHPLC). Column elutant was monitored by an AB Sciex 5600+ triple time of flight mass spectrometer (MS). De novo sequencing was performed on raw MS data (Peaks Studio v7.0 software).

Endogenous and labelled peptides were separated by LC (Agilent 1290 Infinity II HPLC) with a 17 min 0.1% formic acid in acetonitrile gradient. Column eluant was monitored by an Agilent 6495B Triple Quadrupole MS (ESI+ mode). Multiple reaction monitoring was performed with a single quantifier and two qualifier ion transitions. Endogenous peptide identities were confirmed by comparison of retention time, and quantifier/qualifier transition ratios of the respective labelled peptide standards.

Results. 188 unique peptides originating from CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, 3A4 and 3A5, and UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10 and 2B15 were detected. The number of unique peptides detected for each protein ranged between 2 and 19, with a mean of 9.65. By way of example, mean (range) CYP2D6 and CYP3A4 protein abundances in sEV were 192 (79 to 347) fmol/mL and 1094 (713 to 1523) fmol/mL, respectively.

Discussion. This study demonstrated the quantification of CYPs and UGTs in sEVS derived from blood which may be used as a potential source of clinical biomarkers. Additionally, it may complement existing drug probe-based approaches, while possibly circumventing the need for tissue biopsy.

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Determining the pre-analytical instability of beta-Lactam antibiotics in therapeutic drug monitoring samples.

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Introduction. Therapeutic drug monitoring (TDM) is increasingly used to optimise dosing of beta-Lactam antibiotics to improve treatment outcomes. Studies on the stability of beta-Lactam antibiotics to determine sample handling and storage conditions for TDM are conflicting and use inconsistent approaches. As beta-Lactams are inherently unstable, awareness of pre-analytical sample stability is crucial for reliable TDM results. Pre-analytical stability testing typically uses methods designed to confirm stability and methods to define instability are not well established.

Aims. To determine the pre-analytical instability of piperacillin (PIP), tazobactam (TAZ), meropenem (MER) and ceftazidime (CFZ) and to recommend storage and shipping requirements for TDM samples.

Methods. Pre-analytical instability of PIP, TAZ, MER and CFZ were assessed from samples in EDTA and citrate anticoagulated plasma stored at 24°C, 4°C and -20°C and in whole blood (EDTA-anticoagulated) stored at 24°C using an in-house LC-MSMS assay. Instability was determined by non-linear regression analysis of the rate of degradation and 'stability' was defined using the lower limit of the 95th CI of the time to 15% of degradation.

Results. The degradation rates, mean (95% CI), of PIP was 0.023 (0.017-0.030) h^{-1} at 24°C, 0.036 (0.030-0.041) hr^{-1} at 4°C and 0.018 (0.012-0.024) $days^{-1}$ at -20°C and the others were similar. The study found that beta-Lactam antibiotics were 'stable' in EDTA anticoagulated plasma for at least 6 hours at 24°C, 3 days at 4°C and 4 days at -20°C. Stability in citrate anticoagulated plasma and in whole blood was similar to EDTA anticoagulated plasma.

Discussion. The use of mean values to assess sample stability, as per the FDA and EMA guidelines, potentially underestimates analytical error due to sample degradation. We propose stability studies for unstable drugs quantify instability using temperatures, times and other conditions relevant to the clinic. For unstable drugs, we propose quantifying rates of degradation to avoid clinically relevant errors in TDM results. This approach was used to determine storage and shipping conditions for TDM samples for beta-Lactam antibiotics.

Mortensen JS et al (2019) Ther Drug Monit 41:538-543.

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Efficacy and Safety of opioid analgesics for acute pain conditions: An overview of systematic reviews

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Introduction. Opioid analgesics are widely prescribed for acute pain, however there has been no comprehensive overview of the efficacy and safety of opioid analgesics for acute pain conditions.

Aims. To evaluate and summarise the evidence on the efficacy and safety of opioids for acute pain conditions.

Methods. Systematic reviews of randomised placebo-controlled trials were evaluated for evidence on the efficacy and safety of opioid analgesics for acute pain. Two reviewers extracted outcome data and rated the quality of eligible studies. The study outcomes were pain and adverse events. Quality of evidence was assessed using GRADE and the quality of systematic reviews was assessed using the AMSTAR-2 checklist.

Results. Twenty-seven systematic reviews covering 28 indications were eligible. The majority of evidence comes from single dose regimens of single or combination opioids (morphine equivalent dose range 2.25 – 360 mg). There is high to moderate quality evidence that opioids (single or multiple doses) are efficacious for 18 of the 28 acute pain indications we studied including dental surgery, episiotomy, orthopaedic, gynaecological surgery, maternal pain in labour and photorefractive keratectomy. Effect sizes ranged from very small to large. There are ~10 conditions for which evidence is uncertain due to low quality e.g. renal colic. There is moderate quality evidence that opioids are not efficacious for osteoporotic compression fracture and that codeine-containing combination products are not effective for uterine involution after birth. In general, adverse events (AE) rates were higher with opioids compared with placebo. Nausea, vomiting, sedation and dizziness were commonly reported.

Discussion. Opioid analgesics are efficacious for a number of acute postoperative pain conditions however there are some acute conditions for which opioid analgesics are known to be ineffective or are of unclear efficacy.

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Inability of current dosing to achieve carboplatin therapeutic targets in people with advanced NSCLC: impact of systemic inflammation on carboplatin exposure and clinical outcomes

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Introduction. The presence of elevated systemic inflammation in people with advanced non-small cell lung cancer (NSCLC) is associated with significantly shorter survival following carboplatin-based chemotherapy. Inflammation has been shown to reduce hepatic metabolism of chemotherapy drugs causing increased toxicity.

Aims. This study investigated whether alternative clinical factors, such as systemic inflammation, impact carboplatin pharmacokinetics and drug utilisation. The study also examined the ability of current and alternate dosing regimens to meet therapeutic targets.

Methods. Pharmacokinetic data from 72 people with advanced NSCLC treated with carboplatin-based doublet chemotherapy was analysed using non-linear mixed modelling. Covariate analysis was performed to investigate the impact of standard and novel patient characteristics of carboplatin pharmacokinetics. A Monte Carlo simulation of 100,000 representative NSCLC patients evaluated the Calvert formula and novel dosing strategies. The associations between systemic inflammation and chemotherapy drug utilisation and clinical endpoints were also investigated in the pharmacokinetic cohort and an independent cohort of people with advanced NSCLC administered carboplatin-based doublet therapy.

Results and Discussion. In both cohorts, 25-53% of people had elevated systemic inflammation. In the pharmacokinetic cohort, only 16% of patients achieved the desired therapeutic target of carboplatin. Carboplatin exposure was related to renal function, weight and inflammation (platelet-lymphocyte ratio). Increased systemic inflammation was also associated with reduced chemotherapy cycles, reduced response and shorter survival in both cohorts. Simulations of the newly developed model-based carboplatin dosing strategy showed current Calvert dosing was predicted to result in substantial over-exposure in patients with high systemic inflammation.

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Economic evidence for deprescribing medications: a systematic review

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Introduction. Deprescribing research is an expanding area with numerous studies being conducted to assess the health outcomes of deprescribing. However, there is limited data on the economic consequences of deprescribing.

Aims. The objective of this systematic review is to appraise the economic evidence of deprescribing interventions.

Methods. A search was conducted from inception to 11 February 2019 in Embase, Medline, Scopus, DARE, PsychINFO, NHSEED, and CCTR for studies of participants of any age who had their prescribed medication(s) targeted for deprescribing and reported cost outcomes from any perspective. Study characteristics and findings were summarised qualitatively. Study quality was assessed using the Cochrane risk of bias tool and the CHEERS checklist.

Results. Of 2813 screened articles, 26 studies were included: eight aimed to reduce the number of total prescribed medications and 18 examined deprescribing a specific medication or medication class. In studies reducing the number of total medications, the direct costs of medications were compared before and after intervention in seven studies and to a usual care cohort in four, with significant reductions ranging from $8.76USD (AUD$12.93) to $40.30USD (AUD$59.47) per person per month after intervention. In studies deprescribing specific medication classes, the most common included medications impacting the gastrointestinal system (n=11), and TNF inhibitors (n=3). Cost-utility analysis was conducted in three studies, but only one study found deprescribing, of preventative cardiovascular medications when compared with usual care, is likely to be 70-80% cost-effective if the willingness to pay for a gain of 1 QALY is between $26 403.51USD and $39 605.26USD (AUD$38 966.30 and AUD$58 449.44).

Discussion. Preliminary results suggest there is limited, but varied, evidence that deprescribing may reduce costs. Future research considering both the costs and consequences of deprescribing is needed to better understand whether deprescribing is a worthwhile investment

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Substance use in patients with dual diagnosis

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Introduction. Patients with dual diagnosis have one or more mental health disorders and problematic alcohol and/or other drug (AOD) use. After identifying the characteristics of the comorbidities treatment targets both components.

Aims. To review the demographics of patients and the substances identified at the time of confirming the dual diagnosis in a specialist dual diagnosis treatment service.

Methods. A retrospective audit of dual diagnosis client files admitted to a mental health service and referred to a speciality dual diagnosis service between 1 January and 31 August 2019.

Results. Between 1 January and 31 August 2019 149 patients were referred to the dual diagnosis service. Patients' age ranged from 25 to 55 years, and 89 (60%) were male. The substances reported to be used were methamphetamine (ice), cannabis, alcohol, tobacco or 'other' (including GHB, prescription drugs including benzodiazepines, heroin, gambling). Tobacco was used by almost all (87%) patients. Apart from tobacco the primary substance most reported to be used by the 149 patients was ice (47%), followed by alcohol (26%) and then cannabis (21%) with 'other' substances being the primary drug used by 6% of patients. Interestingly, more patients with a diagnosis of Schizophrenia (53%) and Schizoaffective Disorder (61%) reported using ice as their primary drug compared to those patients with Bipolar Disorder (44%). A greater number of patients with Bipolar Disorder reported using cannabis (25%) or alcohol (31%) than those with Schizophrenia (18% and 25% respectively) and Schizoaffective Disorder (17% and 22% respectively). Among the clients with 'other' mental health diagnoses (including Major Depression, Anxiety, Borderline Personality Disorder) similar numbers reported ice (27%), alcohol (29%) and cannabis (26%) as their primary drug used.

Discussion. These results show that, apart from tobacco, ice is the most common primary substance reported to be used by dual diagnosis patients with Schizophrenia, Schizoaffective Disorder and Bipolar Disorder. Clients with 'other' mental health diagnoses did not identify a preferred primary substance. The results highlight the importance of taking a comprehensive AOD history in this patient group because of the diversity of patient profiles.

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Mechanistic Insights into the Synergistic Antibacterial Effect of a Polymyxin B - ivacaftor combination Against Cystic Fibrosis Pseudomonas aeruginosa.

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Polymyxins are used to treat multidrug-resistant Pseudomonas aeruginosa lung infections in cystic fibrosis (CF) patients. As resistance to polymyxins can rapidly emerge in P. aeruginosa with monotherapy, combination therapy is often the only remaining treatment option.

In the present study, we employed metabolomics to investigate the synergistic mechanisms of polymyxin B in combination with the cystic fibrosis transmembrane conductance regulator (CFTR) ivacaftor against polymyxin-susceptible and -resistant P. aeruginosa isolates from the lungs of CF patients. The in vitro synergistic activity of polymyxin B combined with ivacaftor was assessed using checkerboard and static time-kill assays against a panel of isolates from the lungs of CF patients. The metabolomes of two P. aeruginosa strains (PA14 PMB MIC = 2 mg/L; PA-06 MIC = 8 mg/L) were analysed following treatment with polymyxin B, ivacaftor and the combination for 1, 3 and 6 hours.

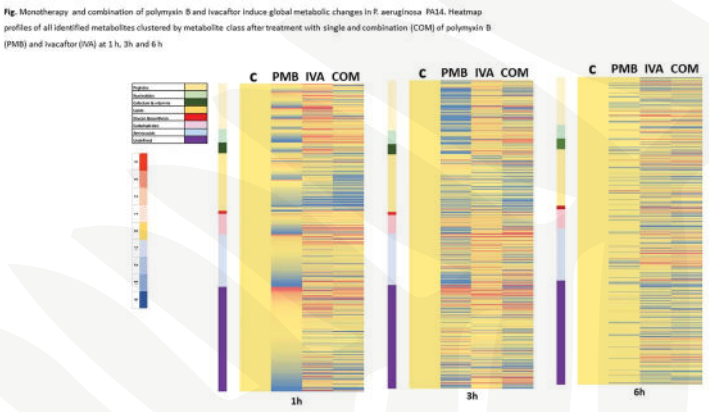

Fig. Monotherapy and combination of polymyxin B and ivacaftor induce global metabolic changes in P. aeruginosa PA14. Heatmap profiles of all identified metabolites clustered by metabolite class after treatment with single and combination (COM) of polymyxin B (PMB) and ivacaftor (IVA) at 1 h, 3h and 6 h

Polymyxin B and ivacaftor were ineffective when used individually. However, when used together, the combination of clinically relevant concentrations of polymyxin B (2 mg/L) combined with ivacaftor (8 mg/L) displayed synergistic killing activity. Polymyxin B monotherapy induced significant perturbations in glycerophospholipid (GPL) metabolism, pentose phosphate pathway, citric acid cycle, pyrimidine ribonucleotide biogenesis, guanine ribonucleotide biogenesis, and histidine degradation pathways in the polymyxin-susceptible strain, and minimal perturbation in polymyxin-resistant strain. The synergy appears to be ivacaftor driven affecting the lipid pathway (Figure).

Overall, these novel findings demonstrate that the disruption of key metabolic features associated with synergistic bacterial killing by the combination against P. aeruginosa in CF.

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SGLT-2 inhibitors: Evidence from observational studies

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Randomised controlled clinical trials (RCTs) have provided evidence on the efficacy of SGLT-2 inhibitors with regard to the reduction of the risk of major cardiovascular and renal events, mainly in patients with high cardio-renal risk. However, RCTs are usually conducted in homogenous and selected patient groups, under strict protocol-driven conditions. Furthermore, RCTs are usually too small to detect rare adverse events (e.g. <1 per 1,000) and too short of duration too assess long-term adverse events such as cancer. Observational studies can complement evidence from RCTs, in particular when studying (rare) adverse events, long-term adverse events, and potentially contribute evidence on effectiveness under 'Real World' circumstances. The main limitation of observational studies is the lack of randomisation and blinding. In particular the study of intended effects can be biased due to confounding by indication. Furthermore, some large-scale observational studies on intended effects (effectiveness) of SGLT-2 inibitors have suffered from immortal and time-lag bias. Several examples of observational studies will be discussed to demonstrate the potential contribution to assess the benefits and risks of SGLT-2 inhibitors.

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SGLT2 inhibitors – Evidence from RCTs

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Sodium-glucose cotransporter (SGLT)2 inhibitors are glucose lowering agents and one of the most exciting drug classes to enter the diabetes market. Designed to treat diabetes, they have been demonstrated to reduce cardiovascular events, particularly heart failure, in cardiovascular outcome trials in people with type 2 diabetes with established cardiovascular disease or multiple cardiovascular risk factors. They also have benefits in reducing the progression of renal disease. Evidence from the main cardiovascular trials will be discussed.

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Emerging adverse drug reactions related to SGLT2 inhibitors

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Sodium-glucose co-transporter protein 2 (SGLT2) inhibitors are a new class of antihyperglycaemic agents, listed in the Pharmaceutical Benefits Scheme in December 2013. SGLT2is have shown promising results not only in reduction in hyperglycaemia but also in reducing hospitalisation for cardiovascular diseases and death. However, there are emerging evidence on adverse drug reactions (ADRs) and adverse events (AEs) not reported in the early randomised controlled trials (RCT). The large CANVAS (CANagliflozin cardioVascular Assessment Study) trial of canagliflozin reported an increase in amputation risk among canagliflozin users compared to placebo. This association has not been reported in other RCTs, but observational studies have reported similar results to the CANVAS trial. A better characterised ADR of SGLT2 inhibitors is euglycaemic diabetic ketoacidosis (euDKA) which is rare but serious ADR. Several case reports including form Australia have been recently published on SGLT2 inhibitors and euDKA. This talk will discuss the recent evidence related to amputation and euDKA risk associated with SGLT2 inhibitors.

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SGLT2 inhibitors in clinical practice

Assoc Prof Matt Doogue, University of Otago, New Zealand

Abstract not available at time of publication

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Precision medicine in lung cancer treatment

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Lung cancer is a major cause of death and suffering in NZ, especially for Māori and Pacific people. New personalised genotype-directed lung cancer treatments have a strong potential to improve lung cancer outcomes. Between 2010 and 2014, PHARMAC funded the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitor (TKI) drugs erlotinib and gefitinib for the treatment of lung cancer, and the Ministry of Health published national guidelines for EGFR mutation testing for identifying patients most likely to benefit from treatment. In late 2019, PHARMAC will fund the Anaplastic Lymphoma Kinase (ALK) TKI drug alectinib for the treatment of ALK-positive lung cancer but no national guidelines for ALK testing are currently available. Our research on the implementation of precision lung cancer treatment in New Zealand has aimed to: 1) evaluate novel strategies for detecting therapeutically targetable mutations across multiple lung cancer genes; 2) generate population-based estimates of the prevalence, demographic profiles, clinical outcomes and use of relevant healthcare services by patients with genetically-defined subtypes of lung cancer, and; 3) understand patterns of the concomitant use of new personalised lung cancer treatments with other medications, as well as identifying combinations of medicines associated with serious adverse events (SAEs) or reduced treatment effectiveness. Results of this ongoing research will be presented. The research has been supported by grants from the Health Research Council of New Zealand (13/981, 15/087 and 19/450).

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Establishment of a therapeutic drug monitoring programme for the treatment of childhood cancer patients in the UK

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Introduction. As we move into the modern era of targeted therapies it is important that alongside a focus on understanding the molecular interactions and target pathways of new anticancer drugs, we also consider clinical pharmacology aspects. The utility of therapeutic drug monitoring (TDM) represents a remarkably under-used clinical tool in oncology, particularly bearing in mind the small margins between sub-therapeutic and toxic drug exposures for many drugs.

Aims. To establish a network of primary treatment centres across the UK actively participating in TDM studies to support treatment individualisation for some of the most challenging childhood cancer patient populations, including pre-term infants and neonates, anephric patients, those receiving high dose chemotherapy and obese patients.

Methods. A national TDM programme of work has now been established in the UK, generating valuable clinical pharmacology data alongside patient characteristics, outcome and response data in defined patient populations. This approach is supported by funding from the NIHR to support the collection/transport of clinical samples from centres around the UK and real-time sample analysis at the NCCPG. We currently have >75 drug assays fully validated according to EMA guidelines, for the quantification of a wide range of anticancer drugs and metabolites.

Results. At the current time approximately 50 patients per annum are receiving TDM-guided treatment for a range of drugs including carboplatin, cisplatin, dactinomycin, vincristine, doxorubicin, etoposide, cyclophosphamide and ifosfamide. Changes to dosing regimens are carried out in >75% of cases, with dose modifications of up to 250% required to achieve therapeutic drug levels, highlighting the potential importance of TDM in a childhood cancer setting. Data generated for carboplatin have recently resulted in the development of national treatment guidelines incorporating TDM dosing for neonates, which are utilised for all children <3 months of age.

Discussion. The establishment of a national TDM programme of work to support dosing decisions provides a real opportunity to generate practice-changing data in these challenging patient populations, where current dosing regimens are routinely based on a limited understanding of clinical pharmacology.

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Comparison of target concentration intervention dosing tools for busulfan in children

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Aim. To assess the ability of model-based dosing tools to estimate busulfan exposure in comparison to clinically used intensive sampling exposure estimation procedure and using limited sampling strategies.

Methods. Data on intravenous bulsulfan dosing from four consecutive days were entered into Bayesian forecasting software, InsightRX and NextDose. The prediction of busulfan cumulative exposure (AUC_{cum}) by each dosing tool was compared to current clinical practice estimation, aiming for pre-defined individualised target exposure for each patient of cumulative busulfan exposure. Estimation performance was tested given several limited sampling strategies.

Results. Thirty-two paediatric patients (0.2-16.5 years) provided a total of 104 daily exposure measurements estimated using seven samples taken per day (full sampling), with 19 patients having four consecutive full sampling days. The median AUC_{cum} (range) achieved by the 19 patients was 72.7 mg·h·L-1 (63.9 – 93.3) based on clinical practice calculations. 16 (84.2%) and 18 (94.7%) patients achieved an AUC_{cum} within ±5 and ±10 $mg{\cdot}h{\cdot}L^{-1}$ of their individual targeted AUC_{cum} set by the treating team, respectively.

Both model-based dosing tools provided acceptable accuracy and precision of cumulative exposure estimations under the tested sampling scenarios. Accuracy ranged from 0.1 - 14.6% using InsightRX and from 3.4 – 7.8% using NextDose. Precision ranged from 19.0 – 32.3% for InsightRX and 8.0 – 9.6% for NextDose.

Conclusion. Model-based dosing software were shown to accurately a nd precisely estimate busulfan exposure un der several limited sampling strategies, which provides evidence for prospective studies to evaluate these tools in clinical practice.

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Predicting severe life-threatening toxicity of fluoropyrimidine drugs

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A key component of treatment of gastrointestinal and breast cancer, 5-fluorouracil (5-FU) is administered either intravenously (IV) or as the oral prodrug, capecitabine. These fluoropyrimidine drugs cause a range of normal tissue toxicities that can lead to dose decreases or delay successive chemotherapy cycles. Of particular clinical concern are gastrointestinal toxicity (diarrhoea and mucositis), hand-foot syndrome and neutropenia. Severe-to-life threatening gastrointestinal toxicity occurs in up to 20% of patients. Dihydropyrimidine dehydrogenase (DPD) catalyses the metabolic clearance of 5-FU. DPD deficiency leads to elevated drug plasma concentrations and increases the risk of toxicity. Rare deleterious *DPYD* gene variants associate with toxicity, however, most toxicity cases do not have an identifiable genetic cause. Assessment of enzyme activity (phenotyping), prior to therapy, has been proposed as an alternative method to identify patients at risk. A challenge test with the endogenous compound thymine (5-methyluracil) could detect aberrant pyrimidine pharmacokinetics in a series of toxicity cases compared with healthy volunteers [Duley et al 2018]. A preliminary case-control study (ACTRN12615000586516) to assess the ability of the thymine test to discriminate cases of severe toxicity from patients who tolerated standard treatment has been undertaken. The data suggest that the thymine challenge test, combined with information on renal function, can detect the majority of those patients who cannot tolerate standard doses of fluoropyrimidine drugs. A larger prospective study of this thymine test is underway (ACTRN12617001109392). This talk will give an overview of these phenotyping studies and also discuss some of the latest genotype and phenotype-guided dose adjustment approaches reported in the literature.

Duley et al (2018) Ther Drug Monit. 40(4):495-502.

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The principles of dosing, response variability, and dose individualisation challenges in children

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Dose selection in children is challenging as many drugs are developed in adults then scaled down for children. The scaling aspect is complex as children undergo non-linear structural and metabolic maturation before reaching adulthood.

Dose requirements vary between individuals due to variability in pharmacokinetic (PK) and pharmacodyanmic (PD) parameters across a population. This between-subject variability (BSV) is comprised of predictable (BSVp) and unpredictable or random (BSVr) components. BSVp can be reduced by accounting for influential covariates on parameter estimates. For instance, the parameter clearance (CL) is significantly influenced by three covariates: body size, functional maturation, and organ function.[1]

Of note, variability in the parameters across a patient population still remains even after accounting for patient covariates. In a review of the reported BSV in PK parameters (quantified using the coefficient of variation percentage), the average CV% for clearance was 40%.[2] Although this refers to PK variability, which is a key source of variability in drug response, complex PD responses (e.g. coagulation) can present significant BSV in PD parameters.[3]

This talk will cover the principles of drug dosing in children and the challenges associated with drug-response variability in this patient group.

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Antibiotic dosing in neonates

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Antibiotic dosing in neonates is challenging due to the high rates of off-label use and lack of high-quality studies evaluating dosing regimens and determining pharmacodynamic targets for neonatal pathogens. Furthermore, the rapid changes in body composition and organ function that occur during the neonatal period affects drug pharmacokinetics, and must be taken into account when determining an optimal dosing regimen. This talk outlines the existing evidence for antibiotic dosing regimens for commonly used antibiotics in neonates with particular focus on vancomycin and meropenem.

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Immunosuppressant dosing in children: learning from children and adults

David K Metz. Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia.

"Children are not just small adults" is an adage well known to paediatricians[1], and failure to recognize differences has historically led to serious consequences. Nevertheless, many (though not all) of the similarities and differences are now much better understood. The argument that children are small adults has been developed and demonstrated in relation to understanding pharmacokinetics from prematurity to adulthood[2]. Data on pharmacodynamic differences between children and adults are less well studied, though there are known differences in susceptibility to toxicities (e.g. related to growth or development). Furthermore, knowledge of the disease process being treated can allow inference of drug effect (i.e. where the process is the same in both children and adults).

None of this obviates the critical need for paediatric trials in drug development, to confirm validity of scaling and inference on PKPD. However, post-marketing, there are conditions for which far more quantitative pharmacological evidence exists in adults than in children (e.g. kidney transplant immunosuppression). It is thus sometimes necessary to "go beyond the 'not just small adults' conceit"[1], so that paediatric clinical practice is maximally informed by all the available data – from children and from adults.

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Predicting time required for complete drug elimination of Infliximab and Adalimumab from infants exposed in utero: A Bayesian forecasting method.

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Introduction. Anti-tumor necrosis factor α (anti-TNF) drugs adalimumab (ADA) and infliximab (INF) are increasingly used in pregnant women with inflammatory bowel disease (IBD). ADA and INF are used to maintain remission and protect the foetus against the impact of active IBD (e.g. abortion, preterm delivery, low birth weight etc.). Infants exposed to anti-TNF drugs in utero have detectable levels of ADA and IFX, which might interact with live vaccination injected to infants 0-12 months after birth.

Aims. Using modelling and simulation method to predict the time required for complete anti-TNF drug elimination for infants, ideally with least number of blood samples collected.

Methods. Population pharmacokinetic models were developed for infliximab and adalimumab respectively, and the population parameters were identified as priors. Bayesian forecasting method was used for predicting time required to undetectable level of individual infant.

Results. A total of 164 infliximab plasma samples from 73 infants and 73 adalimumab plasma samples from 34 infants were used for model development. Bayesian forecasting framework for predicting time to complete elimination of individual infant was developed. Two blood samples (1st sample as cord blood and 2nd as post-delivery vein blood sample) could give satisfied prediction about if the drug at 12 month after birth has been eliminated completely or not.

Discussion. The framework developed could predict time to complete elimination of the drug with only one after-birth blood sample for the infant. This is useful since multiple blood samples are not always easy for infants.

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Investigating the effects of OxR1 antagonism on goal-directed decision making

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Introduction. Orexins are neuropeptides produced by several thousand neurons in the lateral hypothalamus. These neurons project widely through the central nervous system where they bind to regionally selective and largely non-overlapping G-protein coupled receptors: OxR1 and OxR2. Orexins are well known as regulators of the sleep/wake cycle; however, recent investigations into orexinergic modulation of feeding or drug seeking behavior suggest they also play a role in reward processing and decision making, in particular, through OxR1 receptors located in the VTA.

Aims. In this study, we sought to investigate the effects of OxR1 antagonism on goal-directed decision making using an operant probabilistic reversal learning (PRL) task.

Methods. 40 male C57/BL6 mice were dosed daily with an OxR1 selective antagonist (1-SORA-51, 45mg/kg) or vehicle (20% w/v TPGS) prior to performing a PRL task consisting of 5 days of probabilistic discrimination learning, followed by 5 days of reversal learning, both on and off drug in a crossover design. 019

Results. Compared to TPGS control, animals took significantly longer to make decisions in the operant task on drug. We then characterized animal choices using reinforcement learning models consisting of separate learning rates for positive/negative reward prediction errors (RPE) and a perseveration parameter. Animals dosed with 1-SORA-51 show a substantial decrease in positive RPE learning rate compared to TPGS control, with no differences in the negative RPE learning rate.

Discussion. This suggests that OxR1 antagonists do not suppress learning overall, but rather decrease the updating of reward values following positive RPEs selectively. As such, OxR1 antagonists may be of interest in models of abnormal reward processing.

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An evaluation of timed snapshot signalling bias analyses for non-equilibrium systems

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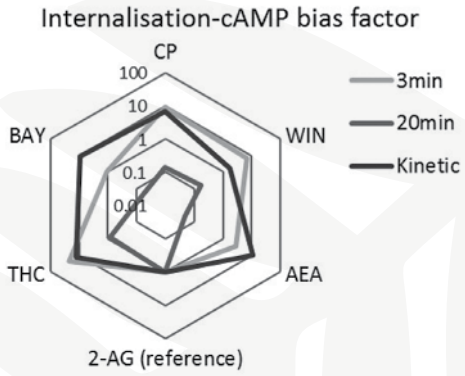
Introduction. Signalling bias describes the ability of ligands to differentially regulate multiple signalling pathways when coupled to a single receptor. Due to their ease of implementation, equilibrium models are often used to quantify signalling bias even when the bio-assay data are not at equilibrium (e.g. due to rapid internalisation). In contrast, a non-equilibrium (i.e. kinetic) modelling approach is robust to the kinetic conditions of the system but is considerably more complicated to perform.



Aim. To evaluate the inference of different timed snapshot equilibrium analyses when compared to a full kinetic analysis.

Methods. Data were available for internalisation, cAMP inhibition and the phosphorylation of ERK (pERK) of the CB_1 receptor. A full kinetic model for CB_1 signalling was developed to simultaneously describe the time-dependent activities across three signalling pathways. Snapshot equilibrium analyses were performed at two time points, 3 and 20 min, and were then compared to the signalling bias results of the full kinetic model (the gold standard for non-equilibrium experiments).

Results. The developed kinetic model adequately described the signalling profiles of the CB_1 receptor. A snapshot analysis at 3 min showed most of the ligands displayed signalling bias towards internalisation over cAMP inhibition; a result that was similar to the bias pattern observed in the full kinetic assessment. In contrast, a snapshot analysis at 20 min demonstrated an opposite pattern with most of the ligands preferring the cAMP pathway. Note that at 20 min, results could not be determined for the pERK pathway response.

Discussion. The inference of signalling bias results from snapshot analyses when applied to non-equilibrium conditions are time-dependent. Application of an equilibrium model at a snapshot in time may yield very different results from the true system bias when performed at different times and should not be used in these circumstances.

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Cigarette smoking cessation does not reverse hippocampal-dependent cognitive impairment in mice.

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Introduction. Chronic obstructive pulmonary disease (COPD) is a major, incurable global health burden and is currently the 4th largest cause of death globally, with cigarette smoking (CS) being the largest causative factor. COPD is characterized by persistent airflow limitation and lung inflammation. It is thought that these inflammatory mediators may 'spill over' into the systemic circulation causing damage to distal organs (e.g. brain) manifesting the neurological comorbidities of COPD resulting in cognitive dysfunction; impairment in learning, and memory, as well as concentration difficulties. Additionally, smokers whom attempt to quit CS are in high risk of relapsing within the first week of cessation, suggesting that the early cessation period is critical in CS withdrawal maintenance. Furthermore, smoking cessation has been associated with worsened performance in memory tasks. However, the mechanisms underlying brain pathology in COPD are unknown.

Aim. To investigate whether hippocampal-dependent behaviour and neuroinflammation is altered upon CS cessation.

Methods. We assessed both spatial (spontaneous Y-maze [sY-maze] test) and working (novel object recognition [NOR] test) memory as well as hippocampal microglial numbers and morphology (ionized calcium binding adaptor molecule-1 immunohistochemistry) in male BALB/c mice exposed to CS (9 cigarettes/day, 5 days a week) for 8 weeks followed by a 10 day CS cessation period.

Results. 8 weeks of CS exposure caused hippocampal-dependent spatial (sY-maze; n = 10-12; $p = 0.012$) and working (NOR; n = 8; $p = 0.004$) memory impairment. In addition, we found that the microglia were activated in the CS mice compared to air-exposed mice (n=8; $p = 0.001$). Interestingly, cessation of CS did not improve memory deficits in spatial (n = 10-12; $p = 0.012$) or working memory (n = 8; $p = 0.004$) to that of air-exposed mice but did reverse the microglial profile to that of air-exposed mice (n=8; $p = 0.001$).

Discussion. CS exposure is sufficient to impair hippocampal-dependent memory, and this coincides with increased microglial activation. However, CS withdrawal does not reverse this memory impairment despite the improved microglia profile. Future research is currently being undertaken to investigate the mismatch between the neuropathology and memory deficits following CS cessation.

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Regulation of ABC lipid transporter expression by oxysterols and cholesterol synthesis intermediates: Implications for Alzheimer's disease

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Introduction. Sterol homeostasis in the brain has been linked to neurodegenerative conditions like Alzheimer's disease. Brain cells express a number of sterol transporters belonging to the ABC transporter family of proteins, including the highly homologous half-transporters ABCG1 and ABCG4. Knockout mouse models accumulate oxysterols and cholesterol synthesis intermediates in the brain, implicating these lipid regulators as transport substrates. In addition, recent reports have implicated ABCG4 in the net export of amyloid-beta across blood-brain barrier (BBB) endothelium. The regulation of these transporters in brain cells is poorly understood and of importance in terms of understanding the disease pathology as well as identifying therapeutic targets. We have previously shown that the post-translational regulation of these transporters is complex and affected by their own substrates (1).

Aims. To investigate transcriptional and post-translational regulatory aspects of ABCG1 and ABCG4 in brain cell models

Methods. ABCG1 and ABCG4 overexpressing cells and brain cell derived cell lines, including neuronal, BBB endothelial and astrocyte cell lines were studied. Cells were cultured in the presence of Liver-X Receptor (LXR) ligands and sterols, including oxysterols and cholesterol synthesis intermediates (complexed to cyclodextrin to deliver equimolar amounts). ABC transporter expression was measure via rtPCR and protein expression via SDS-page.

Results. ABCG4 expression was induced by Liver-X Receptor (LXR) ligands in astrocytes but not in the other cell types, which was contrary to published data suggesting that ABCG4 is not an LXR target. ABCG1 and ABCG4 protein levels was affected significantly by different sterol classes, suggesting that despite extensive homology, these two half-transporters are distinct in terms of their regulation. ABCG4, but not ABCG1 protein was stabilized by cholesterol and cholesterol-synthesis intermediates, in particular lathosterol and lanosterol. ABCG1 protein expression however was more affected by oxysterols, including 25- and 27-hydroxycholesterol, which are potent endogenous LXR ligands.

Discussion. The importance of these findings in the context of neurodegenerative disease will be discussed.

(1) Aleidi *et al* BBA Mol Cell Biol Lipids 2018.

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Idiosyncratic effects of vigabatrin result from gain-of-function $GABA_A$ variants

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Introduction. Developmental and Epileptic Encephalopathies (DEEs) are rare and severe neurological conditions often associated with intellectual disability, developmental delay, autism and movement disorders. Seizures begin in early infancy, and patients are often resistant to antiepileptic treatments. Genetic factors play a major role. Recently, pathogenic variants were identified in the γ-aminobutyric acid type A receptor ($GABA_AR$) that cause DEE.

Methods. Functional genomic approaches not only ascertain pathogenicity of variants but can explain efficacy and adverse effects of drugs. Here we evaluated the functional effects of *de novo* variants in *GABRB3* (p.E77K and p.T287I) and *GABRB2* (p.L255V) genes encoding $GABA_ARs$ in three patients by expressing recombinant human single and double-mutated receptors in *Xenopus* oocytes. Drug responses were assessed using two-electrode voltage clamp methods and were compared to the estimated maximum open probability (Est P_o). Statistical significance of GABA potency at WT and mutant receptors was assessed using a one-way ANOVA and post-hoc Dunnett's test (n= 6) and $p > 0.05$.

Results. Patients with *GABRB3* variants were clinically vigabatrin-hypersensitive, explained by atypical gain-of-function $GABA_ARs$ as identified by a significant 3-5 fold shift of the GABA response to the left. By contrast, benzodiazepines had reduced efficacy for all variants, explaining their reduced benefits and lack of adverse effects in these patients.

Conclusion. The study demonstrates that functional genomics can predict beneficial and adverse anti-epileptic effects.

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The *in vitro* biological effects of Cannabichromene enantiomers and analogues

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Introduction. Over 100 phytocannabinoids have been extracted from *Cannabis spp*., however only Cannabichromene (CBC) has been shown to be a selective CB2 receptor agonist (Udoh et al, 2019). Thus, this phytocannabinoid is pharmacologically interesting because it lacks the psychoactive properties observed with Δ^9-tetrahydrocannabinol which activates CB1 receptors. Due to its therapeutic potential and growing commercial interest, determining CBC active enantiomer and active analogues is important to make them commercially available using a number of biosynthetic and semisynthetic approaches, hence without depending on growing *Cannabis* crops. Here we sought to test these compounds in well-established assays that probe the activation of CB1 and CB2 receptors.

Methods. We used a membrane potential assay (Molecular Devices) to measure potassium channel (GIRK) opening (hyperpolarisation) induced by (±)-CBC, its enantiomers and analogues in AtT20 FlpIn2 cells stably expressing CB1 or CB2 receptors. Responses were expressed as a percentage of the maximum response of a reference non-selective cannabinoid receptor agonist, CP55940 (1µM). Receptor specificity and $G_{i/o}$ involvement were determined by testing the compounds in wild-type (WT) cells and incubating transfected cells with pertussis toxin (PTX).

Results. Highly purified, (±)- CBC produced a selective CB2 activation with a notional pEC_{50} of 5.54±0.15, maximum response of 71±4 % at 30 µM. Most of the activity rested in the (+)-CBC (Mazzoccanti et al, 2017), with a notional pEC_{50} of 5.9±0.1 and response at 30 µM of 78±3% compared to a response of 37±5% for the (-)-CBC (30 µM). CBC analogues had a range of activities, with some unable to activate either CB1 or CB2 receptors, and others having similar responses to CBC. All compounds (10 µM) activated CB1 by less than 15% of the CP55940 maximum. Hyperpolarisation was absent in WT and PTX-treated cells.

Discussion. We were able to confirm that purified CBC enantiomers and CBC analogues selectively elicited a CB2 receptor response in agreement with previous work (Udoh et al, 2019). Interestingly, purified (+)-CBC has a considerably higher activity than (-)-CBC, which may even have no effect, as any effect could be derived from residual (+)-CBC.

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Mazzoccanti G *et al* (2017) Chem Commun (Camb) Nov 14;53(91):12262-12265.

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Responding the WHO 3rd global patient safety challenge: Development of an inter-professional medication safety education programme

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Introduction. Australia is a participant of the WHO 3rd global patient safety challenge. Medication safety is a core principle for all professionals involved in safe and effective management of drugs. Medication management is taught traditionally in silos within health professional degree programs. Inter-professional education enables students from multiple professions to learn core clinical and teamwork skills within authentic clinical teams. Prescribing, medication management and administration provides an exemplar activity for interprofessional education.

Aims. To develop an understanding of individual and shared professional roles and responsibilities to safely prescribe, dispense and administer medicines.

Methods. To minimise the logistical challenge of timetabling across 3 student cohorts, asynchronous blended teaching activities were developed and integrated into 3 Units of Study within MD, B.Pharmacy and B.Nursing degrees, (n=750 students). Three clinical scenarios were designed to illustrate emergency presentations of a patient with myocardial infarction, community-acquired pneumonia and urinary tract infection with comorbid diabetes and kidney dysfunction. Students in Medicine firstly review and complete the prescription of current and new medications prior to review and dispensing by Pharmacy and subsequent simulated medication administration by Nursing 6 weeks later. Quizzes were built into the Canvas learning management system to quantify the student engagement of the medication chart at each stage of the medication cycle and development of a interprofessional de-brief session for all disciplines for reflection on the shared responsibility for medication safety.

Results and Discussion. A framework-based approach to medication management and prescribing skills from both a discipline and inter-professional approach was developed for medical nursing and pharmacy curricula. Plans for pilot testing of the activities and extension from paper to electronic prescribing will be further discussed.

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The third iteration of a foundational unit in a new Pharmacy curriculum.

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Introduction. The Faculty of Pharmacy and Pharmaceutical Sciences at Monash University strategically redesigned the Pharmacy curriculum to better align with the needs of the profession. A key feature of the new Vertical Integrated Masters of Pharmacy degree (VIM) is skill development such that students learn to be critical thinkers, problem solvers, excellent communicators and team players.

Aim. To describe the process undertaken to design, develop and deliver a foundational unit within the VIM degree at the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University.

Methods. Units were purposefully restructured to adopt a student-centred learning approach. Lecture notes were replaced by self-directed online modules which were incorporated into the learning management system (Moodle) by an educational support team of assistant lecturers. In-class didactic lectures were replaced with newly developed Interactive Lectures comprised of active learning exercises *only*. Workshops were also designed with student centred learning in mind.

Results. In 2017, the units were delivered for the first time to a cohort of ~190 1st year Pharmacy students. Both students and instructors were engaged throughout the entire learning / teaching process. Now in the third year of its inception, we report our experience in the initial design, development and delivery of a foundational unit, How the Body Works, as well as the changes that were made to improve the unit in time for the second and third offering in 2018 and 2019 respectively. The impact of these changes were reflected in the teaching evaluation surveys which showed an 8% and 11% increase in the overall unit satisfaction in 2018 and 2019 respectively.

Discussion. Utilising a different teaching approach, new units were developed as part of the new Pharmacy curriculum which focuses on skill development. Feedback obtained from staff and students this year will be used to further develop the unit in 2020.

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Multiple choice questions: Can they assess application of knowledge in Pharmacology teaching?

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Introduction. Application of knowledge is one of the core attributes of graduates from pharmacy schools and related disciplines in Australia and worldwide.

Aims. This study aims to examine the use of multiple choice questions (MCQs) to assess the application and analysis of knowledge in a pharmacology course.

Methods. MCQs were developed and ranked into one of two cognitive levels, based on a modified Bloom's taxonomy, including knowledge recall 'KQ' and application/analysis 'AQ'. MCQs were included in the final exam of the Vet Pharmacology course, then administered to the 1st year Doctor of Veterinary Medicine (DVM) Students. Student performance on MCQs was compared between and within each Bloom's level throughout the Pharmacology course over four years. The differences in the % of students who obtained a correct answer for each level were then compared using Student's t test.

Results and Discussion. A total of 68 DVM students were enrolled in the Pharmacology course in 2019. One hundred MCQs (comprised of 41 KQ and 59 AQ) were included in the exam. The overall average MCQ score on the exam was 70.5%. No significant difference in student performance was observed between recall and application/analysis (66.4% and 73.0%, $p > 0.05$). Similarly, no significant difference in student performance was found between overall average score and recall ($p > 0.05$) or application/analysis ($p > 0.05$). Student performance on MCQs is also compared between 2019 to the previous three years, and the results are to be discussed/,presented in this paper at the meeting.

Conclusion. In summary, well-designed MCQs which target various cognitive levels can be used in pharmacology exams to facilitate assessment of student performance.

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Developing techniques to isolate and characterise individual Russell's viper venom peptides and proteins

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Introduction. Russell's Viper (*Daboia siamensis*) envenoming is frequent and significantly important across several South-East Asian countries. Clinical effects in Russell's viper envenoming vary according to the geographical location, which may be explained by differences in venom composition, and little is known about the venom composition from snakes in this region.

Aims. This study aims to develop techniques to separate and characterise individual venom proteins from Russell's viper venom.

Methods. Venoms of Myanmar and Indonesian origin were separated by cation exchange FPLC, fractions collected and subjected to SDS-PAGE followed by trypsin digestion and tandem mass spectrometry (MS/MS). Identified fragments were matched to known Russell's viper proteins within the UniProt database.

Results. Ion exchange chromatography with crude venom of Myanmar origin has resolved 8 protein peaks and of Indonesian origin has resolved 9 protein peaks. The MS data from Russell's viper venom of Myanmar origin identifies a higher proportion of Kunitz-type protease inhibitors, conversely the Russell's viper venom of Indonesian origin identifies a higher proportion of phospholipase A2 proteins. A small number of peptides were exclusively identified in the MS analysis of Myanmar Russell's viper venom; a Zinc metalloproteinase disintegrin, a Phospholipase A2 and a cysteine-rich secretory protein. Likewise, a small number of peptides were exclusively identified in the MS analysis of Indonesian Russell's viper venom; a Disintegrin and a L-amino-acid oxidase.

Discussion. This is the first proteomic analysis of Burmese and Indonesian *D. siamensis* venom which may assist in elucidating the underlying effects of Russell's viper envenoming in victims of different geographical origins.

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The *in vitro* neurotoxicity of Indian cobra (*Naja naja*) venom: efficacy of antivenom

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Introduction. The Indian cobra (*Naja naja*) is among the 'Big Four' responsible for most human snake envenoming in India. Despite recent proteomic studies indicating the presence of neurotoxins in *N. naja* venom (Dutta et al, 2017), the mechanism of action of the venom and neurotoxins require further investigation.

Aim. To study the *in vitro* neurotoxicity of *N. naja* venom, to isolate and characterise the major neurotoxins, and to determine the efficacy of Indian polyvalent antivenom (IPAV) against the whole venom and the neurotoxins.

Methods. Venom was fractionated by reverse-phase high performance liquid chromatography (RP-HPLC). *In vitro* neurotoxicity of venom and isolated fractions was determined in the electrically stimulated chick-biventer cervicis nerve-muscle (CBCNM) preparation. Isolated toxins were analysed by matrix-assisted laser desorption/ionisation (MALDI-TOF) mass-spectrometry and N-terminal sequencing. The *in vitro* efficacy of IPAV was assessed by prevention and reversal studies i.e. either addition before venom or after venom when the twitches were inhibited by 90%.

Results. Venom (1-10 µg/ml; n=4) caused concentration-dependent inhibition of indirect twitches in the CBCNM and abolished responses to exogenous acetylcholine and carbachol. Three toxins, isolated by RP-HPLC, inhibited indirect twitches (0.1-1 µg/ml) and responses to exogenous acetylcholine and carbachol. MALDI-TOF mass spectrometry analysis indicated that the toxins contained intact masses of 6916 Da, 7020 Da and 7808 Da, respectively. N-terminal sequencing of the toxins indicated close structural homology with previously isolated short- and long-chain post-synaptic neurotoxins (Barber et al, 2013). IPAV prevented the neurotoxic effects of venom and the toxins but did not reverse the neurotoxicity of the venom.

Discussion. This study demonstrated that *N. naja* venom and its major neurotoxins are post-synaptic in nature can be neutralized, but not reversed, by IPAV.

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Dutta S et al (2017) J Proteomics 156:29-39

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Multitask deep learning for metabolism-aware strain-specific assessment of Ames mutagenicity.

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Introduction. Traditional in-silico assessments of chemical mutagenicity based on Ames bacterial reverse mutation assay data are singly tasked with classifying chemicals in a binary manner; i.e. mutagenic positive or negative. Closer inspection of the in-vitro Ames protocol reveals the use of (a) multiple Salmonella typhimurium strains for different operon locations of frameshifts and base-pair substitutions, and (b) incubation of chemicals in S9 rat liver homogenate for metabolic activation of promutagens. Integration of this data into Ames models enables mechanistic interpretation of the currently binary assessment of mutagenicity. However, developing single-task models for each strain/metabolism combination is inefficient and can result in worse predictive performance for data-scarce strains.

Aims. Herein, we investigate a deep learning approach to simultaneously model multiple strain/metabolism combinations to generate a mechanistically-interpretable fingerprint profile of Ames mutagenicity for input chemicals.

Methods. ~5000 chemicals tested on up to eight strains with and without S9 incubation were extracted from OECD QSAR Toolbox. A multitask neural network simultaneously modelling all 16 strain/S9 combinations will be developed and compared to 16 control single-task models based on balanced accuracy and ROCAUC (confidence intervals calculated via bootstrapping). Held-out testing will be performed on clinical therapeutics [1] and textile dyes [2].

Results. Preliminary testing of a multitask neural network modelling the eight unmetabolised strains saw highest average ROCAUC across all eight tasks compared to traditional single-task models. We expect further increases in predictive performance from the addition of the eight metabolised strains to the multitask neural network.

Discussion. Simultaneous modelling of the 16 combinations using a multitask approach reduces development costs compared to 16 separate models, and improves predictions as strain-specific QSARs are now shared within the model. The model prediction is a 16-bit fingerprint that can enable mechanistic assessment for regulators by revealing whether a chemical mutates by frameshifts or base-pair substitutions and with or without metabolic activation.

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[2] Bruschweiler and Merlot (2017) Regul Toxicol Pharmacol 88:214-226.

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Targeting the NLRP3 inflammasome as a novel approach to treat pulmonary hypertension

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Introduction. Pulmonary hypertension (PH) has no cure and high mortality; elevated pulmonary arterial pressure, vascular remodelling, immune cell infiltration and right ventricular (RV) hypertrophy lead to RV failure and death. The NLRP3 inflammasome-generated inflammatory cytokines interleukin (IL)-1β and IL-18 are elevated in PH patients.

Aims. To utilise a pharmacological approach to investigate the potential contribution of the NLRP3 inflammasome to PH pathogenesis in two aetiologically distinct murine models of the disease.

Methods. PH severity was assessed in gold-standard murine model Sugen/hypoxia (SuHx) and secondary-PH bleomycin model (BLM). Interventions: NLRP3 inflammasome inhibitor MCC950 (10mg [low] or 20mg [high]/kg/day; sc), current therapy sildenafil (SILD; 30mg/kg/day; oral) or combination therapy. Data presented as mean±SEM.

Results. Disease measures included RV systolic pressure (RVSP), (RV/left ventricle [LV] + septum [S]) and lung weight (LW) to body weight (BW) ratios, Martius Scarlet Blue (MSB; fibrosis) and α-smooth muscle actin (α-SMA; vascular muscularisation) staining. All markers of disease severity were increased in SuHx and BLM mice compared to normoxic (NmOx) or saline controls, respectively. RVSP was unaffected by low dose MCC950 but was significantly attenuated with combination therapy (NmOx 27.3±0.4mmHg vs. SuHx 44.2±1.0mmHg; n=16-22; P<0.01; SuHx+MCC950+SILD 38.7±0.9mmHg vs. SuHx; n=15-22; P<0.01). Additionally, preliminary data revealed a trend for a reduction with high dose MCC950 39.6±5.0mmHg (n=3). Pulmonary vascular muscularisation assessed by α-SMA was unchanged by MCC950. MCC950 ameliorated RV hypertrophy (RV/LV+S) in SuHx mice (NmOx 2.5±0.04 vs. SuHx 3.6±0.07 vs. SuHx+MCC950 3.2±0.09; n=14-20; P<0.05). Further, MCC950 attenuated pulmonary fibrosis, demonstrated by reduced LW/BW ratio and diminished MSB staining (BLM+MCC950: 33±3% vs. BLM: 47±3%; n=5; P<0.05).

Discussion. MCC950 reverses RV hypertrophy and lung fibrotic burden in experimental PH, independently of pulmonary pressure and pulmonary vascular remodelling, these data suggest that targeting the NLRP3 inflammasome may provide a novel adjunct to current pulmonary vasodilator therapy.

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Influenza A virus provokes maternal vascular dysfunction, inflammation and intrauterine growth restriction.

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Introduction. Influenza A virus (IAV) infection during pregnancy, drives severe maternal illness and foetal complications, such as fetal growth restriction (FGR), through enigmatic mechanisms despite a lack of vertical transmission. Although studies have identified foetal hypoxia to play a significant role in FGR and neurodevelopment, it remains unclear whether maternal IAV infection induces foetal hypoxia and through what mechanism.

Aim. To examine the role of the innate and adaptive immune system on maternal vascular function following IAV infection.

Methods. Eight-to-twelve-week old time-mated pregnant (E12 gestation) C57BL/6 mice were infected intranasally with IAV (HKx31; 10^4 PFU) or with PBS (n=6-8 per group). Mice were euthanized 3 and 6 days post-infection for analysis of aortic viral burden, and maternal vascular immune profile by qPCR and flow cytometry. Maternal thoracic aorta vasodilation to Ach and SNP were assessed via wire myography. Offspring were analysed for pro-inflammatory and hypoxic markers in placenta and foetal brain via qPCR. Placental and foetal weights were also recorded.

Results. IAV infection during pregnancy resulted in an exacerbated systemic inflammation and viral dissemination into the aorta. This was associated with the elevated expression of adhesion molecule (VCAM1), toll like receptor (TLR)7, pro-inflammatory (IL-6) and anti-viral mediator (IFN-γ) in the vascular wall ($p<0.05$). There was also an influx of $Ly6C^{low}$ and $Ly6C^{high}$ monocytes, neutrophils, CD4 and CD8 T cells to the aortas of IAV-infected mice. Vascular reactivity analysis revealed a significant and dynamic alteration in the maternal vascular landscape (~50-60% impairment in relaxation to ACh ($p<0.05$) during IAV infection. In the offspring, maternal IAV infection resulted in placental and foetal brain hypoxia, as well as intrauterine growth restriction ($p<0.05$).

Discussion. These results indicate that IAV infection during pregnancy drives a significant cardiovascular event in pregnant mothers, which likely suppresses critical blood flow to the placenta and foetus resulting in hypoxia, concomitant intrauterine growth restriction and adverse foetal outcomes.

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Real-world efficacy and safety outcomes of imatinib treatment in patients with Chronic Myeloid Leukemia: an Australian experience

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Introduction. Tyrosine kinase inhibitors have revolutionised the treatment of chronic myeloid leukaemia (CML), but a large proportion of patients still experience treatment-limiting toxicities or therapeutic failure.

Aims. To investigate real-world prescribing and outcomes of imatinib in patients with CML in Australia.

Methods. A retrospective cohort study of patients with CML commencing imatinib between 2001-18 was conducted at two Australian hospitals. Demographic characteristics, prescribing patterns, efficacy and tolerability outcomes were extracted from medical records. Overall survival (OS), progression-free survival (PFS), event-free survival (EFS) and major molecular response (MMR) were evaluated using Kaplan-Meier methods and Cox proportional hazard regression. The incidence rate (IR) of adverse-drug reactions (ADR) was evaluated using negative binomial regression.

Results. 86 patients (median age 56 years [interquartile range 42-66], 59% male, 74% European ancestry) received 89 imatinib treatments. 71% of imatinib treatments required dose modifications (IR 78 per 100 person years (py), 95%CI 57-109). Drug cessation was required in 70% of treatments, due to ADR (50%), poor response (31%) and relapse or disease progression (13%). Estimated OS, PFS and EFS at 5 years were 94% (95%CI 88–100), 93% (95%CI 87-99) and 76% (95%CI 66–88), respectively. EFS was inferior for patients who were poorly adherent or diagnosed in accelerated or blast phase ($P<0.05$). Median time to MMR was 12 months (95%CI 7–21). ADR that resulted in modification or cessation of imatinib occurred at an IR of 221 per 100 py (95% CI 46–345]. Severe ADR had an IR of 152 per 100 py (95%CI 106-221). Pre-existing cardiovascular or pulmonary disease, uncontrolled hypertension, East Asian ancestry, intermediate/high Sokal score, age > 80 years, and first line use were associated with a higher ($P<0.05$) ADR IR.

Conclusions. The efficacy of imatinib in a real-world cohort is comparable to clinical trial results, but with a higher incidence of ADRs and subsequent dose modifications and cessations. Baseline patient demographic and disease characteristics could identify patients more likely to experience treatment-limiting toxicities and inform initial dose and drug selection in patients with CML.

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Identifying and prioritising patients at high-risk of medication harm using risk prediction models

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Introduction. Inpatient medication harm occurs in approximately 7% of patients, with up to 50% thought to be preventable. A predictive model for identifying patients at risk of harm would provide a systematic approach to prioritisation for multidisciplinary medication management. Aims. To develop and validate an Australian risk prediction model, and to validate the predictive performance of two European risk models. Methods. A retrospective cohort study was conducted in general medical and geriatric patients consecutively admitted to a quaternary hospital, over 6-months. Medication harm events were identified using ICD-10 codes, and the hospital's incident management database. Sixty-eight variables, including medications and laboratory tests, were extracted from the hospital's digital databases. To develop the risk model, univariable preselection was used to identify significant variables ($P \leq 0.10$), which were included in multivariable logistic regression analysis. Two European models; The Brighton Adverse Drug Reaction Risk (BADRI) Model (Tangiisuran et al.) and Trivalle's Geriatric Risk Score (Trivalle et al.) were validated in the same patient cohort. Variables were entered into both models and the patients' risk was calculated, and compared with actual outcomes. Model performance was evaluated using area under the receiver operative characteristic curve (AuROC). Results. The study cohort included 1982 patients (median age 74 years), of which 136 (7%) patients experienced ≥1 event(s). The Australian risk model included ten variables: length of stay, new admissions, ≥ 8 medications, serum sodium < 126 mmol/L, INR > 3, use of anti-psychotic, antiarrhythmic and immunosuppressant medications, prior allergy and arterial or venous thromboembolism. Internal validation produced an AuROC of 0.70 (95% CI: 0.65-0.74). The predictive performance for both European models was reduced, with AuROC of 0.63 for the BADRI and 0.60 for Trivalle's model. Discussion. The Australian risk model demonstrated better predictive performance compared with the European models. Next steps include incorporating the new model as part of a digital priority dashboard, and evaluating the clinical impact of the dashboard on clinician workflow and patient outcomes.

Tangiisuran B, et al. (2014) PLoS One.9(10):1-10.
Trivalle C, et al. (2011) Eur Geriatr Med. 2(5):284-289.

247

The M2 macrophage-derived chemokine, CCL18 is elevated in hypertension and promotes vascular fibrosis

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Introduction. M2 macrophages contribute to vascular fibrosis and stiffening in hypertension and may mediate these actions via release of the pro-fibrotic chemokine, CCL18. The role of CCL18 in hypertension and vascular fibrosis has not been investigated.

Aims. This study aimed to investigate the association between plasma CCL18 levels and hypertension in humans, identify vascular targets of CCL18 and explore its ability to promote fibrosis.

Methods. Plasma CCL18 levels from normotensive (SBP: 119±2 mmHg), essential (155±3 mmHg) or resistant (156±5 mmHg) hypertensive patients were measured by ELISA. Human aortic adventitial fibroblasts (AoAFs) and endothelial cells were treated with the pro-fibrotic agent TGF-β (10 ng/ml) or CCL18 (3-300 ng/ml) for 3-72h or 7d, respectively. In human AoAFs, expression of pro-collagen I, mature collagen I and α-SMA and were measured (qRT-PCR, Western blotting). Endothelial-mesenchymal transition was measured via VE-cadherin and α-SMA protein expression.

Results. Plasma CCL18 levels were 48% higher in patients with resistant hypertension as compared to normotensive subjects (64.5 vs 43.5 ng/ml; n= 14-20, $p<0.05$). In human AoAFs, TGF-β caused a 2-fold increase in protein expression of collagen I (24h, $p<0.01$) and α-SMA (24-72h, $p<0.05$). CCL18 (300 ng/ml) did not change α-SMA expression, but increased the protein expression of pro-collagen I by 2-fold (24h; n= 7-9, $p<0.01$), and elevated mature collagen I by 3.6-fold (72h; n= 7-9, $p<0.05$). In human aortic endothelial cells, CCL18 (10 ng/ml) increased α-SMA (1.5-fold, n= 6-10, $p<0.05$) and showed a trend to decrease VE-cadherin (n=4).

Discussion. Resistant hypertension is associated with elevated plasma CCL18 levels. CCL18 targets adventitial fibroblasts and endothelial cells in the vascular wall to promote collagen synthesis and endothelial-mesenchymal transition, respectively. As such, therapeutic targeting of CCL18 may serve as a novel approach for the treatment of hypertension-associated vascular fibrosis.

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Chronic cigarette smoke exposure causes cognitive impairment and neuroinflammation in mice

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Introduction. Chronic obstructive pulmonary disease (COPD) is a highly morbid and irreversible disease characterised by persistent respiratory symptoms primarily caused by cigarette smoking. Many patients with COPD manifest with other medical conditions (i.e. comorbidities) including cognitive dysfunction (CD). Studies have shown that up to 60% of COPD patients suffer from CD, including learning and memory impairments. However, the mechanisms underlying CD in COPD are unknown.

Aim. To investigate whether chronic cigarette smoke (CS) exposure causes neuroinflammation and impairs hippocampal-dependent cognition in mice.

Methods. Male BALB/c mice were exposed to either room air (sham) or CS (9 cigarettes per day, 5 days a week) for 24 weeks. After 23 weeks of CS-exposure, hippocampal-dependent behavioural tests (novel object recognition [NOR] and Y-maze) were conducted to assess working (NOR) and spatial (Y-maze) memory. Mice were culled following 24 weeks of CS-exposure and brains were excised to assess the numbers and morphology of microglia and astrocytes (using Ionised calcium-binding adapter molecule-1 (Iba-1) and Glial fibrillary acidic protein (GFAP) staining respectively), in order to determine if CS-exposure may cause neuroinflammation.

Results. Chronic CS-exposure impaired working memory (NOR) when compared to sham mice (n=8-10, $p<0.05$). In addition, CS-exposure caused a significant reduction in microglial number in both the hilus and CA3 regions of the hippocampus (n=8, $p<0.05$). Furthermore, microglia were significantly more activated in the CA3, subgranular and molecular regions of the hippocampus (n=8, $p<0.05$). CS-exposed mice also showed a reduction in astrocyte density within the CA1 (n=8, $p<0.01$), hilus and the subgranular (n=8, $p<0.05$) hippocampal regions.

Discussion. Chronic CS-exposure for 24 weeks impairs working memory. This impairment is attributed to a suppression of hippocampal microglial number, increased microglial activation and a reduction in hippocampal astrocyte density. Future research is necessary to investigate this altered neuroinflammatory profile as a potential mechanism for the observed memory deficits in COPD.

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Mesoscopic approaches for brain networks: Pharmaceutical and electroceutical insights

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The excitation-to-inhibition ratio in ongoing neuronal activity of various brain areas, including the hippocampus, is homeostatically balanced over time. We recently discovered that mouse hippocampal sharp wave/ripple oscillations serve as intrinsic events that trigger long-lasting synaptic depression and contribute to a day-night neural activity balance. Our findings are consistent with the role of slow-wave states in refining memory engrams by removing recent memory-irrelevant neuronal activity. In my presentation, I explain our findings with the research backgrounds and will try to hypothetically extend their significance to a clinical dimension. I may also extend my talk to recent topics about machine learning.

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PBPK-IVIVE: The Trojan Horse that Re-injected Mechanisms Back into Models

Prof Amin Rostami-Hodjegan, University of Manchester, UK

Classical descriptive models of observed concentration–time data using purely statistical/mathematical models may or may not contain some elements that refer to biological mechanisms. Wide-spread use of drug-independent `system´ information in expanding these models is a concept that distinguishes quantitative systems pharmacology (QSP) type of models from the previous set. However, building QSP models requires a series of drug-dependent parameters that are usually, but not exclusively, measured in vitro. Forward translational of such data (in vitro – in vivo extrapolation, IVIVE) approaches only became acceptable once they were incorporated into the older discipline of physiologically-based pharmacokinetics (PBPK). The presentation outlines how the IVIVE made its way to modelling via PBPK and argues that the PBPK that we talk about today is no longer the one that was traditionally recognised by pure focus on blood flows and partitioning to tissues!

PBPK was only acted as Trojan Horse to bring IVIVE and mechanisms into modelling drug kinetics!

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Allopurinol at the coal face – can we really get to target with an old drug?

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Allopurinol is the most commonly used urate lowering therapy. Despite the fact it has been used for decades many people fail to achieve current treatment goals. In recent years' newer therapies have become available but the cost, availability and adverse event profiles mean allopurinol will remain a first line therapy for some time. Understanding the adverse event profile of allopurinol, how clinicians can minimise the risk of the rare but serious allopurinol hypersensitivity syndrome and still achieve target serum urate with allopurinol is therefore important.

Recent data on how allopurinol can be used in a treat to target fashion as well as potential predictors of response will be presented.

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Model-based dosing in patients with gout: The curious case of allopurinol

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The dosing of allopurinol would appear to be anything but curious. Standard doses of 200-300mg daily were commonly prescribed in the past and have been used in recent comparative clinical trials for newer urate-lowering agents. Dose reduction in patients with renal impairment is not unexpected for a drug with a renally-cleared active metabolite (oxypurinol). The recent finding that maintenance dose requirements vary by 6-9 fold is not particularly unusual either. What is curious however is that dose reduction in patients with renal impairment results in a dramatic reduction in efficiacy [ref], even though the plasma concentrations of oxypurinol are normalised within a presumed 'therapeutic range'. This seemingly paradoxical curiosity is the focus of the model-based analysis described here. The aims of the research programme are to determine the factors that predict the dose-response of allopurinol, to explore the target oxypurinol concentrations that optimise urate-lowering response, and to predict dose requirements to achieve serum urate targets. Allopurinol dose-response was found to be predicted by renal function, body size, diuretic use, and ABCG2 genotype. The oxypurinol concentrations associated with therapeutic response were found to be a moving target, determined mainly by differences in renal function. Dose requirements were driven mainly by diuretic use and body size. Renal function and ABCG2 had only a modest impact on dosing. A revised dosing guideline to achieve a serum urate ≤ 0.36 mmol/L based on simulations from our predictive model will be introduced.

Dalbeth N (2006) J Rheumatol 33:1646-50.

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Off indication medicines and novel strategies with potential to treat gout

Prof Anthony Rodgers, UNSW Sydney, The George Institute for Global Health, UNSW Medicine, Australia

Abstract not available at time of publication

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Strategies To Improve Outcomes for People with Gout: eMed Approaches

Prof Richard Day, UNSW Sydney, UNSW Medicine St Vincent's Clinical School, Australia

The prevalence of gout is increasing in spite of effective medicines. Many studies confirm that poor understanding of the condition and its treatment correlates with treatment failure. Men, predominately afflicted, perceive there is stigma associated with the label, 'gout'. This interferes with seeking help. Core information required is that maintaining serum urate below 0.36 mmol/L will eliminate recurrent attacks. Also, if adherence is poor, attacks of gout will return. Prescribers, predominately general practitioners, need to appreciate that attacks are likely after initiating urate lowering therapy (ULT), almost always allopurinol. This risk is reduced by commencing with a low dose of allopurinol and increasing the dose every few weeks until target urate is achieved. Concomitant colchicine for 6 months can reduce the risk of attacks further.

Adherence rates to allopurinol are 50% or less, worse than any other chronic illness. Efforts to enhance primary care physician's knowledge to help them manage gout successfully has had limited impact. In contrast, nurses trained to educate and support the management of patients with gout has been very effective. However, this approach is too resource intensive to replicate at scale. Will 'personalised', eHealth interventions, that have shown some promise in improving management of some other chronic disorders, be able to support patients with gout adhere to their ULT?

Apps for smart phones are now available to assist people with chronic health conditions. Their design needs to accommodate the barriers and enablers to adherence to ULT identified through qualitative studies of patients with gout. Individual feedback of serum urate may represent an important enabler of adherence to ULT, and this facility is possible with apps.

Harnessing mobile apps to support patients managing their chronic illnesses represents an important opportunity to enhance health outcomes. Rigorous, patient-centred and driven development is critical. These tools also require careful evaluation. Gout is an excellent 'test bed' to develop effective apps for chronic disease self-management.

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Amnion epithelial cell therapy for stroke: Translation of experimental findings into a Phase I clinical trial

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Stroke accounts for more than 10% of deaths worldwide, and over a third of survivors are left with major neurological impairment. The need for new and effective therapies for stroke is therefore clear and urgent. There is increasing interest in cell therapy as another treatment modality in stroke, particularly for patients who are unable to receive endovascular clot retrieval or thrombolysis therapies, or for whom standard treatment has failed. Human amnion epithelial cells (hAECs) are non-immunogenic, non-tumorigenic, anti-inflammatory cells normally discarded with placental tissue following childbirth. We have shown that hAECs provide neuroprotection in three models of cerebral ischemia in mice as well as in non-human primates. hAEC therapy attenuated infarct growth and/or promoted functional recovery, even when administered 1-3 days after the onset of stroke (Evans et al, 2018). The mechanisms of action involve modulation of the immune response to minimise further injury in the peri-infarct region, the so called 'inflammatory penumbra'. Unlike neuroprotection with unimodal action, multipotent hAECs have the ability to adapt their actions on the peri-infarct region over time, appropriate to the pathophysiological state of the target tissue. To translate these preclinical findings, we have commenced a Phase 1 dose escalation trial to assess the safety of allogeneic hAECs in stroke patients with a view to providing an evidence platform for future Phase 2 efficacy trials (Phan et al, 2018). The trial is registered with Australian New Zealand Clinical Trials Registry (ACTRN12618000076279). Our protocol involves a modified 3+3 dose escalation study design with additional components for measuring magnetic resonance signal of efficacy and the effect of hAECs on immunosuppression after stroke. Patients are eligible if they have ischemic stroke in the territory of the middle cerebral artery, present within 24 h of stroke onset and are not eligible for thrombolysis or clot retrieval, aged 18-85 years, and have NIHSS stroke severity score of 6-15. Currently 3 patients have received 2 million cells/kg, with no adverse events for up to 7 months.

Evans MA et al (2018) Stroke 49:700-709.
Phan TG et al (2018) Frontiers in Neurology Jun 7;9:198.

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Tailoring metabotropic glutamate receptor 5 activity with biased allosteric modulators for neurodegenerative disorders

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Introduction. Metabotropic glutamate receptor subtype 5 (mGlu5) modulates neurotransmission in response to the major excitatory neurotransmitter glutamate and is a promising target for multiple neurological and psychiatric disorders. Allosteric modulators that interact with sites distinct from glutamate, are of significant interest due to their ability to fine-tune receptor activity and potential for greater subtype selectivity. Positive allosteric modulators (PAMs) enhance, whereas negative allosteric modulators (NAMs) inhibit glutamate responses. Discovery programs commonly screen and classify ligands based on potency determinations, which lack sufficient rigor and can result in misinterpretation of activity.

Aims. We propose that allosteric modulators from diverse chemotypes differentially influence mGlu5 activity. We are developing novel chemical tools to decipher mGlu5 function in native cells.

Methods. We apply rigorous analytical methods to investigate multiple measures of mGlu5 activation to dissect the functional consequences of allosteric modulation in recombinant and primary cells using high-throughput assays for Ca^{2+} mobilisation, inositol phosphate accumulation and ERK1/2 phosphorylation.

Results. We have found that allosteric modulators can differentially activate and/or modulate distinct signalling pathways, referred to as biased agonism and biased modulation, respectively. For both mGlu5 PAMs and NAMs, distinct bias profiles can be linked to *in vivo* efficacy and may be predictive of adverse effect liability. We have developed novel first-in-class chemical tools that selectively label native receptors.

Discussion. Ultimately, our work will provide a better understanding of the mechanisms driving on-target therapeutic versus adverse effects and provide a framework for future rational discovery campaigns for biased modulators that can fine-tune receptor activity to the pathway level.

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The delta-subunit selective $GABA_A$ receptor modulator, DS2, improves stroke recovery via an anti-inflammatory mechanism.

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Introduction. Inflammatory processes are known to contribute to tissue damage in the central nervous system (CNS) across a broad range of neurological conditions, including stroke. Gamma amino butyric acid (GABA), the main inhibitory neurotransmitter in the CNS, has been implicated in modulating peripheral immune responses by acting on $GABA_A$ receptors on antigen-presenting cells and lymphocytes.

Aims. Here we investigated the effects and mechanism of action of the delta-selective compound, DS2, to improve stroke recovery and modulate inflammation.

Methods and Results. We report a decrease in NF-κB activation in innate immune cells over a wide concentration range *in vitro*. When DS2 was administered to LPS-challenged mice *in vivo*, we observed a significant decrease in the production of several pro-inflammatory cytokines. Following a photochemically-induced motor cortex stroke, treatment with DS2 at 0.1 mg/kg from 1 hour post-stroke significantly decreased circulating tumor necrosis factor (TNF)-α and interleukin (IL)-17 levels, reduced infarct size and improved motor function in mice. Free brain concentrations of DS2 were found to be lower than needed for robust modulation of central $GABA_A$ receptors and were not affected by the presence and absence of elacridar, an inhibitor of both P-glycoprotein and BCRP. Finally, as DS2 appears to dampen peripheral immune activation and only shows limited brain exposure, we assessed the role of DS2 to promote functional recovery after stroke when administered from 3-days after the stroke. Treatment with DS2 from 3-days post-stroke improved motor function on the grid-walking, but not on the cylinder task.

Discussion. These data highlight the need to further develop subunit-selective compounds to better understand change in GABA receptor signaling pathways both centrally and peripherally. Importantly, we show that GABA compounds such as DS2 that only shows limited brain exposure can still afford significant protection and promote functional recovery most likely via modulation of peripheral immune cells and could be given as an adjunct treatment.

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Molecular Pharmacology of Cannabinoid Receptor 2, a Promising Immunomodulatory Target

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Introduction. Cannabinoid receptor 2 (CB_2) is a G protein-coupled receptor which is expressed on immune cells both in the periphery and centrally. Activation is thought to exert predominantly immunosuppressive effects and CB_2 is considered a promising target in a range of disorders with immune system involvement including neurodegeneration and stroke. However, to date the majority of the studies in this field have been performed on cell lines, rodent models, or stimulated primary cells.

Aims. We aim to study CB_2-mediated signalling and functional outcomes in primary human immune cells under conditions which closely mimic their *in vivo* state.

Methods. Human primary mononuclear leukocytes (PBMC) were isolated from healthy donors and cultured for a limited time (up to 6h) prior to assay in media with 10% foetal bovine serum. cAMP, p-ERK, p-p38, p-CREB, p-Akt and p-JNK were measured with LANCE or AlphaLISA kits (Perkin Elmer), and secretion of interleukins 6, 8 and 10 was measured by cytometric bead array (BD Biosciences). Coupling of signalling mediators to effector pathways has been probed via the use of selective signalling pathway inhibitors.

Results. We have observed an unexpected cAMP signalling profile, wherein classically-expected $G\alpha_i$-mediated inhibition of cAMP synthesis occurs concurrently with $G\alpha_s$ mediated stimulation of cAMP synthesis, a phenotype not previously observed for CB_2. We have further characterised downstream signalling and measured an immunologically relevant functional outcome – secretion of interleukins 6, 8 and 10. We observe functional selectivity between CB_2 ligands and find that the particular signalling fingerprint elicited influences the cytokine secretion profile.

Discussion. These findings impact the potential for targeting CB_2 in a range of conditions, including neuroinflammation. This work provides not only insight critical for furthering CB2-targeted drug development, but highlights wider considerations for GPCR signalling studies and model validity.

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Using patient simulation in the lecture theatre to introduce “real-life” scenarios

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A problem experienced by many medical schools is how to deliver meaningful, interactive and impactful teaching of clinical pharmacology to large classes in a lecture theatre format. Newcastle University has campuses for medicine in the UK and Malaysia, with class sizes of around 350 and 150 students respectively. Active learning pedagogies are required to engage and stimulate such large class sizes, with many teachers turning to technology enhanced learning strategies, such as the use of apps, multimedia and online polling.

A second challenge with large class sizes is how to provide sufficient early clinical exposure, necessary to help the students integrate their basic pharmacology knowledge with clinical practice. Simulation, with either role players and/or virtual patient simulators such as SimMan, allows students to apply their clinical pharmacology knowledge in a safe and standardised learning environment. Simulation can complement clinical exposure, since clinical exposure alone does not guarantee the learning of core competencies.

In response to the above challenges, we designed clinical scenarios for enactment in the lecture theatre combining role-players, SimMan and online student polling [1]. We built scenarios around students’ clinical pharmacology and clinical skills learning outcomes, in particular around the basic clinical and pharmacological management of the acutely unwell patient. The simulations allow exploration of communication and patient management skills, while students voting on the appropriate management for SimMan ensures active engagement. The use of props, such as oxygen masks and nebulisers, together with the patient monitor that displays the patient’s vital signs adds to the realism and learning opportunities.

In the symposium, I will demonstrate some of the techniques we use and suggest alternatives for faculty without access to a high-fidelity patient simulator.

1. Guilding. Choose your own story: combining interactive voting technology and high-fidelity patient simulations in the lecture theatre, for large group preclinical medical education. BMJ Simulation and Technology Enhanced Learning 2016;2:47-48.

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Using simulations to develop proficiency in the application of pharmacokinetic concepts

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The concept of "Start by looking at your data." is familiar to all pharmacometricians, and the pedagogical approach of encouraging students to visualise data through drawing of graphs is well-accepted. There is now a strong precedent for enhanced learning *via* use of interactive dynamic visualisation tools.[1] Aligned with this, we are using dynamic simulations to develop work place readiness in both the pharmacy and pharmaceutical sciences undergraduate degrees at Monash University. Our current approach, established for 10 years in teaching of postgraduate (PhD) courses nationally and internationally, uses the simulation software Berkeley Madonna in a small class environment.

An important aim is to enhance the students' understanding of the physiological basis and impact of PK and PK/PD parameters on concentration-time profiles and implications for dosage regimens and drug development. In addition, the approach is being applied to address a gap that was identified with traditional purely calculations-based teaching of these areas, namely the link between proficiency in the calculation of PK parameters, and students' understanding of how/why the ability to apply PK and PK/PD concepts is important in their future work places.

This has been achieved through simple tailoring of scenario context in order to make them more tangible and relevant to the students' future careers. Pharmacy students are presented with patient scenarios in a community or hospital pharmacy and work through the application of PK and PK/PD concepts to solve case-based problems (*e.g.* how does dosing need to be changed based on patient characteristics such as renal function). Scenarios for pharmaceutical science students are contextualised around a drug discovery/development program seeking to understand compound PK to guide medicinal chemistry or support formulation/product development.

Looking beyond our established use of Berkeley Madonna, changes in student learning behaviour and the need for flexible delivery calls for the development of visualization tools that can be accessed by students "on demand" and without the need for dedicated software. To this end, interactive visualizations using Shiny for the R programming language (building on concepts described previously[2]) offer an attractive opportunity.

Moreau D (2015) Front Psychol 6:342, 2. Wojciechowski J et al (2015) CPT Pharmacometrics Syst Pharmacol 4: e21.

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Using Patient Simulation in the Clinical Learning Environment

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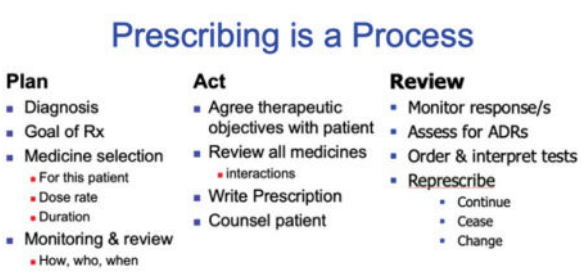
Introduction. Prescribing errors are common and junior doctors struggle with all aspects of prescribing. Prior learning can reduce some errors. Electronic prescribing and administration (ePA) systems are replacing paper prescribing. The apprenticeship model provides opportunities for authentic experiences in the clinical environment. This can be augmented by simulation.



Aim: For junior doctors to be able to prescribe medicines correctly on their first day of work.

Methods. Medical students are provided with access to the hospital electronic prescribing and administration (ePA) system. They have access to both training and production environments throughout their 4th to 6th years of training. The ePA training environment is used for a one hour prescribing simulation session during the 6th (final) year of training. Students were surveyed before and after the simulation session. The ePA production environment is used to prescribe medicines to patients under supervision during clinical attachments. Use is audited weekly.

Results. Following greater than a year of open access to the ePA system and before simulation 36% of students reported being confident or very confident using the electronic prescribing system. After the simulation session this increased to 100% (36/36). While using the hospital ePA system under supervision approximately 1% (125/1226) of prescriptions were signed by students in error. None of these (0/1226) were clinical errors (not intended by the clinical team).

Discussion. A one hour supervised simulation session significantly increased student confidence in using an ePrescribing system. Open access to ePA systems leads to inappropriate signing by students, but does not lead to prescribing errors. Student access to ePA systems facilitates clinical learning. ePA systems can be audited to monitor student (and clinician) prescribing patterns.

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Real-time simulation to help students become more confident and reflective practitioners

Stephen Duffull, School of Pharmacy, University of Otago, New Zealand

Therapeutic decision-making is a cognitive-based skill that is required by practitioners who are involved in initiation, changing or discontinuing a medicine for a patient. It is a cornerstone of clinical pharmacology training across all the health professions who contribute to care of patients using medicines. A difficulty in learning this skill is the requirement for the student to enact their decision with a patient but do so in a safe and effective way.

In this presentation, I will explore the use of a real-time simulation based game (SimPHARM) as a tool to engage students with therapeutic decision-making. SimPHARM is an autonomous, cloud-based simulation application in which each student gets to make observations, order lab tests, ask the patient questions and make therapeutic interventions to treat a virtual patient in order to attain their therapeutic goals. The simulation is real-time (1 minute of the virtual patient's time = 1 minute of real-time). This way students get to experience the consequences of their decision as they would naturally unfold in clinical practice. Cases can be run from 1 hour to 4 days and the student interacts at times that suit their daily routines. Each case diverges as it is played so no two student's experiences are necessarily the same.

In our pharmacy training programme at Otago we introduced SimPHARM in 2018. Once a student is assigned a case, the student completes this in their own time and then (in a flipped classroom style) debriefs in small groups with a facilitator. At the debrief students discuss: what happened in their cases (normalisation of experiences), their key decision and justification and their reflection about whether their decision(s) met their therapeutic target goals. Here reflection is used to drive the learning process. In our experience, more than 98% of students undertaken the SimPHARM case prior to the debrief session, students indicate that they feel like a practitioner, display evidence of authentic behaviours and respond well to reflection-driven learning.