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Poster Abstracts

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Naturally occurring polymorphisms in cardiac bitter taste receptors (T2Rs) dramatically alter receptor function.

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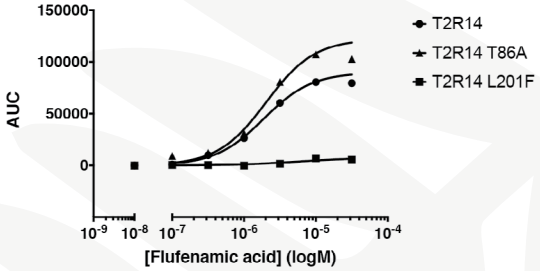
Introduction: G protein-coupled receptors (GPCRs) are important regulators of cardiovascular physiology. Bitter taste receptors (T2Rs) are a new class of GPCR that are expressed in a wide range of extra oral tissues e.g. brain and lungs. Our laboratory has previously reported the expression of T2Rs in the heart, and have since seen drastic cardio-depression upon application of known bitter ligands to electrically paced explanted heart tissue. T2Rs are highly polymorphic, but the role of these cardiac T2R polymorphisms are not well characterised.



Aim: To investigate the activation and signalling profiles of naturally occurring polymorphisms in cardiac specific T2Rs.

Methods: Polymorphisms, identified using UCSC Genome Browser, were introduced into TAS2R expression plasmids using site directed mutagenesis. HEK293T cells were transiently transfected with either wildtype or mutant T2Rs, and ligand-stimulated intracellular Ca2+ mobilisation was measured using a fluorometric imaging plate reader (FLIPR).

Results: Variants for T2R46: L228M, T2R14: T86A and L201F and T2R50: C203Y have a penetrance of 42%, 2%, 2% and 40%, respectively. Compared to the most common variants (referred to as wild-type) some polymorphisms resulted in profound changes in receptor-mediated activation (e.g. T2R14 L201F and T2R46 L228M having a completely abrogated signal in response to their respective ligands, flufenamic acid and andrographolide).

Discussion: Some naturally occurring polymorphisms are able to dramatically alter receptor-mediated activation. It is predicted that the normal physiological function of these receptors within the cardiovascular system would be altered due to the effects of some of these polymorphisms. Furthermore, non-functional polymorphisms may provide the unambiguous controls necessary to attribute changes in cardiac physiology seen in human tissue to T2R dependent signalling.

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The trends and opinions of Australian clinicians on the use of antihyperglycemic agents in heart failure patients

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Introduction: Metformin (MET) use in heart failure (HF) patients is contraindicated in Australia, in contrast to other countries. However, anecdotal evidence shows that MET is prescribed in HF patients. Further, recent trials of MET in combination with SGLT2 inhibitors (SGLT2i) show improved HF outcomes. In response, use of combination SGLT2i and MET has increased, however there is no data investigating the opinions of clinicians on the use of AHG in HF patients.

Aim: To explore the use of, and influences on, antihyperglycemic (AHG) prescribing by Australian cardiologists and endocrinologists in the management of T2D in HF patients.

Methods: Cardiologists and endocrinologists at St Vincent's Hospital, Sydney (SVH) participated in a survey and semi-structured interview. Interviews were transcribed and analysed for themes. A longitudinal, retrospective audit (at least two visits 2014-19) of HF patients with T2D on MET (n=50) at SVH was also conducted. Data collected included AHG prescription details, demographics and relevant pathology (incl. UEC, HbA1c, NT-ProBNP)

Results: All clinicians (n=15) reported that the best T2D therapy for HF patients was SGLT2i with MET. Key influences on prescribing decisions were renal function and cardiovascular benefit studies. MET use in HF was considered safe. However, 30% (4/15) reported that the occurrence of euglycaemic ketoacidosis in SGLT2i is understated. Several clinicians highlighted the need for HF specific T2D AHG guidelines. In the audit, most patients were male (92%) over the age of 60 with mild kidney disease (n=50). Prescriptions of AHG at visit one compared to final visit (median = 232 days) revealed MET alone (18% vs 15%); dual MET + insulin (9% vs 0%), SGLT2i (18% vs 45%) or GLP-1 (4% vs 0%); and triple MET + Insulin + DPP4i (9% vs 0%), SGLT2i + insulin (0% vs 9%) or SGLT2i + DPP4i (0% vs 5%). Clinical notes stated reasons for most SGLT2i addition was due to glycaemic control and HF benefits.

Discussion: Clinicians continue to prescribe MET for HF patients with T2D. However, published research heavily influences prescribing decisions, reflected in the increased prescriptions of SGLT2i in patients with HF and T2D in the current patient cohort. These outcomes support larger cohort studies into the long-term safety of SGLT2i in HF patients.

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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 Ly6Chi and Ly6Clo 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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Defining the characteristics of an alternate mouse model of type-2 diabetes (T2D)-induced cardiomyopathy

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Introduction: Diabetes is associated with an increased risk of heart failure due to altered structure and function of the heart, commonly termed diabetic cardiomyopathy. Mice are the most widely utilised for experimental studies investigating diabetes, however most are genetic models of diabetes (e.g. *db/db, ob/ob*) and do not reflect the human condition due to their altered leptin signalling.

Aims: We sought to characterise the altered cardiac, kidney and liver structure and function in a mouse model of T2D-induced cardiomyopathy incorporating the combination of low dose streptozotocin (STZ) and high fat diet.

Methods: Male 6-week-old FVB/N mice received three daily i.p. injections of STZ (55mg/kg/d) followed by 26 weeks of high-fat diet (42% energy from lipids, Speciality Feeds) to induce T2D. Non-diabetic (ND) mice received vehicle and normal chow diet. Blood glucose, pulsed-wave Doppler and tissue Doppler echocardiography, body composition, plasma insulin, markers of myocardial, kidney and liver structure, remodelling and function, were assessed.

Results: T2D mice exhibited significantly increased body weight and fat mass, increased blood glucose and plasma insulin, and albuminuria ($P<0.05$ for all). Heart weight and left ventricular weight, as well as kidney weight, were unaltered by T2D, however liver and spleen weights were significantly increased ($P<0.05$). Markers of cardiac fibrosis (MMP9, PAI-1), hypertrophy (β-myosin heavy chain) and apoptosis (p-P46 JNK) were increased in T2D hearts ($P<0.05$ for all). At endpoint, there was clear evidence of diastolic dysfunction in terms of reduced E/A and e'/a' ratios, with prolonged deceleration time and isovolumic relaxation time ($P<0.05$ for all). In the kidney, mesangial area and collagen IV gene expression were increased in T2D mice ($P<0.05$). In the liver, plasma markers of liver function (ALT, AST), expression of ProCol3, NAFLD activity score and steatosis grade were all increased with diabetes ($P<0.05$ for all).

Discussion: This study reveals that the combination of low-dose STZ and high-fat diet mimics several clinical features of T2D, and importantly, produces robust diastolic dysfunction at 26 weeks which is a characteristic of patients with T2D-induced cardiomyopathy.

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Diabetes impairs the contribution of nitric oxide (NO•) but not nitroxyl (HNO) to endothelium dependent relaxation in rat carotid arteries

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Introduction: Endothelial dysfunction is a major risk factor for the vascular complications of diabetes. HNO, the one electron reduced and protonated form of NO•, is resistant to scavenging by superoxide, but the fate of HNO in diabetes associated endothelial dysfunction is unknown.

Aim: To assess how diabetes affects the role of endogenous NO• and HNO in endothelium-dependent relaxation.

Methods: Diabetes was induced in male Sprague Dawley rats with high fat diet (HFD) for 14 weeks and low dose streptozotocin (STZ; 35 mg/kg ip/day for 2 days), whilst control rats were fed standard chow and administered citrate vehicle. At study end, rats were anaesthetised and carotid arteries collected to assess responses to the endothelium-dependent relaxant ACh using myography. Using either the combination of Ca-activated K channel blockers TRAM34 (1 µmol/L) and apamin (1 µmol/L) or the NO synthase inhibitor, L-NAME (200 µmol/L) and ODQ, a soluble guanylate cyclase inhibitor (10 µmol/l), the contribution of endothelium-dependent hyperpolarisation and NOS-derived nitrogen species to relaxation was assessed. L-cysteine (3 mmol/L), a selective HNO scavenger and hydroxocobalamin (HXC; 100 µmol/L), a NO• scavenger were used to distinguish between NO• and HNO-mediated relaxation.

Results: At study end, diabetic rats had lower body weight than control rats (541±19 vs. 619±21 g, n=19, 16, P<0.01) and higher blood glucose (25.4±1.2 vs. 6.5±0.2 mmol/l, P<0.01). In carotid arteries from diabetic rats, ACh-induced relaxation was significantly impaired (pEC50: control 7.24±0.17, n=13; diabetes 6.72±0.18, n=16, P < 0.05). The ACh-evoked relaxation was abolished by L-NAME plus ODQ but not affected by apamin plus TRAM34, indicating that NOS-derived nitrogen species are the predominant endothelium-derived vasodilators in control and diabetic rat carotid arteries. In control and diabetic carotid arteries, maximum relaxation to ACh was significantly decreased by L-cysteine whereas HXC only inhibited relaxation in control, indicating diabetes impaired the contribution of NO• but not HNO.

Discussion: Both NO• and HNO contribute to endothelium-dependent relaxation in carotid arteries but only the NO•-mediated relaxation is impaired in diabetes while the HNO component of relaxation is preserved.

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Pharmacological activity of perhexiline enantiomers in an isoprenaline model of myocardial damage

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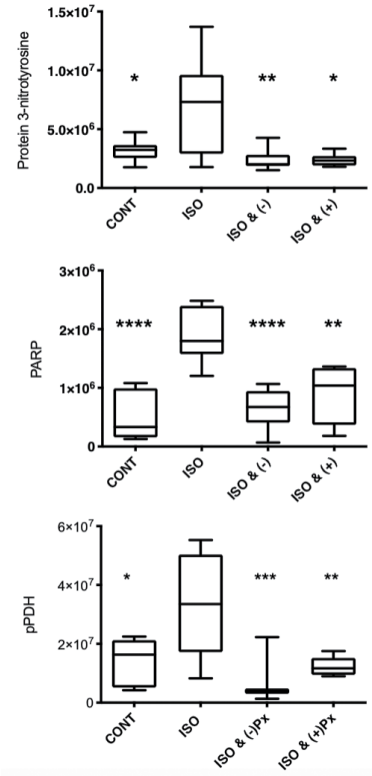
Introduction: At physiological concentrations, reactive oxygen species (ROS)/nitrogen species (RNS) play a role in maintaining healthy myocardial cellular metabolism. However, excessive ROS leads to formation of peroxynitrite RNS, and consequent poly-ADP ribose polymerase-1 (PARP) activation, ATP depletion and myocardial damage. Perhexiline (Px) improves myocardial energetics and inhibits NOX2 activation, a major source of myocardial ROS. We have previously shown that both (+)-Px and (-)-Px enantiomers inhibit NOX2 activation, with IC50 values of 1.6 and 1.2 µM, respectively.

Aims: To determine the effects of (+)- and (-)-Px on protein nitrosylation, PARP and pyruvate dehydrogenase (PDH) during isoprenaline (ISO) induced myocardial damage.

Methods: ISO (50mg/kg i.p.) was administered to Dark Agouti rats following 2 weeks of dosing with vehicle or 200mg/kg p.o. (+)- or (-)-Px (n=5-9). Hearts were assessed histologically 48h later for inflammatory infiltrate and necrosis. Nitrosylated proteins, PARP, total and phosphorylated PDH (pPDH) were determined by western blotting.

Results: ISO caused myocardial inflammation and necrosis, which was attenuated by (-)-Px (P<0.05) but not (+)-Px. ISO increased nitrosylated proteins, PARP, and pPDH compared to controls, and both (+)- and (-)-Px attenuated these effects (Figure, ANOVA *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to ISO). There were no changes in total PDH.

Discussion: Both Px enantiomers have beneficial effects with respect to ISO-induced nitrosative stress and PARP expression, potentially through inhibition of NOX2 activation. The attenuation of both PDH inactivation (pPDH) and PARP expression may help maintain cellular ATP. There may be a greater benefit of (-)-Px with respect to maintaining PDH activity.



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Adiponectin receptor gene therapy as a treatment for diabetic cardiomyopathy in mice

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Introduction: Adiponectin is a cardioprotective adipokine that is reduced in serum in type 2 diabetes (T2D). Impaired cardiac adiponectin signalling in diabetes is in part, due to a downregulation of both adiponectin receptors (AdipoR1 and R2). Adiponectin signalling has been shown to protect against cardiac remodelling and lipotoxicity in several cardiac pathologies, however its cardioprotective influence in T2D-induced cardiomyopathy has not been investigated.

Aims. To target cardiac adiponectin signalling utilising cardiac-selective adiponectin receptor adeno-associated viral (AAV) gene therapy as a treatment for diabetic cardiomyopathy in mice *in vivo*.

Methods: T2D was induced in 6-week-old male FVB/N mice via streptozotocin (55mg/kg per day, 3 consecutive days, i.p.) followed by a high-fat diet (42% calories by lipid), controls received citrate vehicle and placed onto standard chow diet. After 18 weeks of diabetes, left ventricular (LV) function was assessed via Doppler echocardiography under anaesthesia (ketamine/xylazine/atropine, 80/8/0.96mg/kg). Mice were then administered AAV6 gene therapy containing either AdipoR1, AdipoR2, AdipoR1+R2, or null empty vector (2x1011 vector genomes, i.v.), and followed for 8 weeks. At end point, LV remodelling, diastolic function, lipotoxicity, and adiponectin signalling were assessed.

Results: At study end, blood glucose and HbA1c were elevated ($P<0.01$). LV diastolic dysfunction was confirmed after 18 weeks of diabetes by a reduced E/A ratio and increased deceleration time ($P<0.05$ for both). At endpoint, AdipoR2-AAV modestly improved LV diastolic function as demonstrated by a reduction in deceleration time ($P=0.055$). Gene expression of adiponectin receptors was unaltered with AAV gene therapy. Interestingly, LV collagen deposition was reduced in diabetic mice treated with both AdipoR1 and AdipoR2 gene therapy, compared to diabetic null-treated mice ($P<0.05$). Gene expression for the inhibitor of lipotoxicity, acyl-CoA oxidase 1, was increased in diabetic mice treated with AdipoR1 or AdipoR2 AAV gene therapy (2.2±0.1 and 1.9±0.2 fold increase, respectively, $P<0.05$).

Discussion: The therapeutic potential of elevated cardiac adiponectin signalling in the context of T2D-induced cardiomyopathy may improve LV function and remodelling associated with increased cardiac lipotoxicity.

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The effects of cannabidiol on the contractions of rat isolated mesenteric arteries and other muscle tissues.

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Introduction: Cannabidiol is a non-psychotropic phytocannabinoid derived from Cannabis sativa plant. It is used clinically to treat spasticity in patients with multiple sclerosis and seizures in children with treatment-resistant epilepsy syndromes. Given the increasing use of cannabidiol, it has become essential to pharmacologically characterise the effects of cannabidiol at clinically relevant concentrations on cardiovascular and other tissues.

Aims. To assess the effects of cannabidiol on the contractions of rat isolated mesenteric arteries to various contractile agents in comparison to its effects on the contractions of three rat muscle tissue types: smooth, cardiac and skeletal.

Methods: Myographs were used to evaluate the effects of cannabidiol (0.3-3 µM) on the contraction of small mesenteric arteries (200-350 µm i.d.) to various contractile agents: endothelin-1 (ET-1), 5-hydroxytryptamine (5-HT), U44619, methoxamine and arginine vasopressin (AVP), as well as to study the effects of cannabidiol (10-100 µM) on the contractions of bronchi (smooth muscle). In organ baths, the inotropic and chronotropic effects of cannabidiol (10-100 µM) were evaluated in left and right atria (cardiac muscles), respectively, in addition to its effects on the contractions of vasa deferentia (30-100 µM; urogenital smooth muscle) and the hemidiaphragm (30 µM; skeletal muscle).

Results: The contractions of mesenteric arteries to all agents were significantly inhibited by cannabidiol 3 µM. Methoxamine- and 5-HT-induced contractions were also inhibited by cannabidiol 1 µM. The order of sensitivity of the different contractile agents to inhibition by cannabidiol was: 5-HT>methoxamine>U46619>AVP=ET-1. The contraction of bronchi and hemidiaphragm to carbachol and nerve activation, respectively, was unaffected by cannabidiol. Cannabidiol did not shift the noradrenaline contraction curve of the vas deferens, however a higher concentration (100 µM) decreased the maximum contraction. In the cardiac muscle tissues, cannabidiol (10-30 µM) caused negative inotropic effects on electrically stimulated left atria, while having no chronotropic effects on the right atria.

Discussion: Our findings suggest that the relaxant effects of cannabidiol at clinically relevant concentrations are restricted to the isolated mesenteric arteries in comparison to the skeletal, cardiac and non-vascular smooth muscles. Further investigations are required to establish whether: i) the effects of cannabidiol may be observed in other arteries, both resistance and larger arteries, ii) to elucidate the mechanism(s) of action of cannabidiol and iii) investigate the haemodynamic effects of cannabidiol in rat.

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Gene delivery targeting cardiac O-GlcNAc improves mitochondrial function in a model of diabetic cardiomyopathy

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Introduction: Sustained increases in O-GlcNAc post-translational modification have been implicated in the development of diabetic cardiomyopathy and mitochondrial dysfunction. Two enzymes regulate this modification; O-GlcNAc transferase (OGT) facilitates the addition, and O-GlcNAcase (OGA) facilitates O-GlcNAc removal. Whether modulating O-GlcNAc modification is therapeutic in heart disease has not been established.

Aims. To investigate whether cardiac-targeted OGA gene delivery improves mitochondrial function in the context of diabetes.

Methods: Diabetes was induced in 6wk-old male mice via streptozotocin (55mg/kg/day i.p./day, 5 days). After 8wks of diabetes, a single i.v. injection of recombinant adeno-associated virus-6 (rAAV6)-OGA (2x1011 vg or 1x1012vg) or control vector was administered, and mice were assessed followed for a further 8wks. In parallel, human iPSC-derived cardiomyocytes were maintained In-vitro in low glucose (LG) or high glucose (HG) conditions and transduced with rAAV6-OGA. Mitochondrial and glycolytic activity were assessed in cells, via the Seahorse Extracellular Flux Analyser.

Results: In-vivo Complex V ($P<0.06$ vs control) and Complex III ($P<0.05$ vs control), proteins involved in oxidative phosphorylation, are reduced in the diabetic heart. This was not evident in rAAV6-OGA treated mice. In-vitro analyses of human iPSC-derived cardiomyocytes showed no difference in glycolytic function between cells maintained in control and HG media in terms of glycolysis, glycolytic capacity, glycolytic reserve and non-glycolytic acidification. However, mitochondrial respiration assays revealed a trend for HG-induced reduction in basal respiration, ATP production and maximal respiration ($P=0.07$, $P<0.05$, $P<0.05$ respectively vs control). These impairments were not evident in rAAV6-OGA-treated HG cells, with a significant improvement in maximal respiration ($P<0.05$ vs HG).

Conclusion. Administration of rAAV6-OGA limits mitochondrial dysfunction in the diabetic heart, suggesting that alteration of O-GlcNAc signalling plays a vital role in mitochondrial function.

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Reduced adenosine A2B receptor protein expression in adult rat primary cardiac myofibroblasts.

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Introduction: Adenosine protects tissues and cells during tissue damage or hypoxic-ischaemic conditions where its role becomes important to counter metabolic stress and cellular energy imbalance [1]. Adenosine influences the remodelling of cardiac tissue and its receptors are expressed on fibroblasts [2]. Fibroblasts transform into myofibroblasts during cardiac damage and they are key effector cells of tissue remodelling.

Aims. To determine the adenosine receptors present in adult rat cardiac fibroblast and myofibroblasts and the effects of adenosine receptor agonists on wound healing rates and cell proliferation in adult rat primary cardiac fibroblasts.

Methods: Adult rat primary cardiac fibroblasts were isolated from Wistar rat (8 weeks) ventricles and once established in cell culture transformed into myofibroblasts using TGF- β1 (5 ng/ml). The adenosine receptor subtypes were identified in rat cardiac fibroblast and myofibroblast cells using Western blot analysis. The effects of adenosine receptor agonists and antagonists were tested on rat isolated primary cardiac fibroblasts undergoing wound healing and cell proliferation assays.

Results: Both rat isolated primary cardiac fibroblast and myofibroblast cells expressed all four adenosine receptor subtypes (A1, A2A, A2B, A3). There were no differences in the protein expression of the adenosine receptor subtypes A1, A2A and A3 receptors between fibroblast and myofibroblast cells ($p>0.05$), however a significant decrease in the protein expression of adenosine A2B receptors was observed in myofibroblast cells ($p<0.05$). No significant effects were observed for all the adenosine receptors agonists or antagonists tested on adult cardiac primary fibroblasts in the wound healing or cell proliferation assays ($p>0.05$).

Discussion: All the adenosine receptor subtypes are present in rat isolated primary cardiac fibroblast and myofibroblast cells. Protein expression of the adenosine A2B receptor was reduced in the myofibroblast cells. The adenosine receptors are not involved with the wound healing process and the role for the adenosine receptors in cardiac tissue remains to be elucidated.

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2. Grden M et al. Biochem Biophys (2006) 455: 10-17.

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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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Investigating the impact of high glucose on adiponectin signalling in human iPSC-derived cardiomyocytes

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Introduction: A reduction in cardiac expression of the cardioprotective adipokine, adiponectin, its receptors (AdipoR1 and AdipoR2) and its downstream signalling molecules, AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor α (PPARα), are associated with diabetes. Downregulation of this pathway is also associated with increased pathological cardiomyocyte hypertrophy and lipotoxicity - common features of diabetic cardiomyopathy. We sought to investigate whether high glucose conditions *in vitro* could mimic the phenotype of diabetic cardiomyopathy, and the potential for activation of adiponectin signalling to ameliorate these pathologies.

Aims. To determine whether stimulation of adiponectin signalling by the small-molecule adiponectin receptor agonist, AdipoRon, attenuates diabetic cardiomyopathy in induced pluripotent stem cell (iPSC)-derived cardiomyocytes.

Methods: Cardiomyocytes (n=7 passages), differentiated from the human iPS-Foreskin-2 cell line, were incubated in low (11mM) or high (33mM) glucose conditions (to mimic the diabetic milieu) and then treated with vehicle (0.05% dimethyl sulfoxide, DMSO), 25μM or 50μM of AdipoRon for 24 hours. Gene and protein expression of AdipoR1, AdipoR2, AMPK and PPARα, and markers of cardiac remodelling and lipotoxicity were measured.

Results: No changes in gene or protein expression of AdipoR1, AdipoR2 and AMPK were observed with incubation in high glucose conditions, nor with AdipoRon treatment under low glucose conditions. In high glucose conditions, treatment with 50μM AdipoRon increased PPARα expression (1.77±0.51 fold-increase, P<0.05). Expression of the lipotoxicity marker, acetyl-CoA carboxylase (ACC), was modestly upregulated by the combination of high glucose and 50μM AdipoRon (1.48±0.26 fold-increase, both P<0.05). AdipoRon treatment (50μM) downregulated the hypertrophic marker, B-type natriuretic peptide, in low but not high glucose conditions (0.13±0.06 fold-decrease, P<0.05).

Discussion: Although high glucose conditions did not induce the changes associated with diabetes *in vivo*, adiponectin receptor agonism tended to increase PPARα and ACC expression. Development of an improved *in vitro* model of diabetic cardiomyopathy is required to further investigate the potential for adiponectin signalling to blunt lipotoxicity.

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Investigating the mechanisms of the inhibitory effects of relaxin on the NLRP3 inflammasome in myofibroblasts

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Introduction: Recombinant human relaxin (RLX) can exert its anti-fibrotic effects by impeding the pro-fibrotic influence of cytokines such as TGF-β1 and interleukin (IL)-1β. This is achieved through its ability to inhibit measures of NLRP3 inflammasome priming and activation (which is a known producer of IL-1β and IL-18) in primary human cardiac myofibroblasts (HCMFs) *in vitro* and mice with isoproterenol (ISO)-induced cardiomyopathy *in vivo*. However, the mechanisms involved in the myofibroblast NLRP3 inflammasome-inhibitory effects of RLX remain unknown.

Aim: To determine the signal transduction pathways by which RLX inhibits NLRP3 inflammasome priming and activation in primary HCMFs *in vitro.*

Methods: Primary human cardiac fibroblasts (HCFs; ScienCell, CA, USA) were stimulated with TGF-β1 (T; 5ng/ml) alone to undergo differentiation into myofibroblasts or with T+LPS (100ng/ml)+ATP (5mM) (T+L+A) for 8h to stimulate NLRP3 inflammasome activation. Dose-response studies of pharmacological inhibitors of the ATP receptor, P2X7R (A438079; 0.05-0.5μM); nNOS (N-propyl-L-arginine/NPLA; 0.05-0.5μM); toll-like receptor (TLR)-4 (TAK-242; 0.05-0.5μM); reactive oxygen species (n-acetylcysteine/NAC; 1-5μM) and caspase-1 (Ac-YVAD-CHO; 0.05-0.5μM) were performed in T+L+A-stimulated HCMFs to determine the most suitable concentration of each inhibitor that did not affect NLRP3 inflammasome priming or activation after 8h. Then T+L+A-stimulated HCMFs were treated with RLX (100ng/ml) ± A438079 (0.05μM); NPLA (0.1μM); TAK-242 (0.1μM); NAC (1μM); Ac-YVAD-CHO (0.05μM); or the AT2R antagonist, PD123319 (0.1μM) for 8h. Changes in TLR-4, NLRP3, IL-1β and IL-18 were determined by Western blotting.

Results: T+L+A-stimulated HCMFs had significantly increased protein expression of TLR-4, NLRP3, pro-IL-1β and pro-IL-18 after 8h (all by 20-40%; all $p<0.05$ vs T alone; n=6). However, RLX administration significantly inhibited these measures after 8h (all $p<0.05$ vs T+L+A; n=6). These inhibitory effects of RLX were abrogated by co-administration of NPLA (0.1μM) or TAK-242 (0.1μM) (both $p<0.05$ vs T+L+A+RLX; n=6); while its effects in the presence of the other inhibitors are currently being evaluated.

Discussion: RLX signals through a nNOS-TLR4-NLRP3 inflammasome-dependent mechanism in HCMFs to inhibit the pro-fibrotic influence of the TGF-β1/IL-1β and TGF-β1/IL-18 axes, as part of its anti-fibrotic effects.

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The mitochondria-targeted methylglyoxal sequestering compound, MitoGamide, is cardioprotective in the diabetic heart.

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Introduction: Methylglyoxal, a by-product of glycolysis and a precursor in the formation of advanced glycation end-products, is significantly elevated in the diabetic myocardium. Therefore, we sought to investigate the mitochondria-targeted methylglyoxal scavenger, MitoGamide, in an experimental model of spontaneous diabetic cardiomyopathy.

Methods: Male 6-week-old Akita or wild type mice received daily oral gavage of MitoGamide or vehicle for 10 weeks. Several morphological and systemic parameters were assessed, as well as cardiac function by echocardiography.

Results: Akita mice were smaller in size than wild type counterparts in terms of body weight (WT-V 29.9 ± 1.2 g vs Akita-V 22.8 ± 0.9 g, $P<0.001$) and tibial length (TL: WT-V 17.3 ± 0.1 mm vs Akita-V 16.7 ± 0.1 mm, $P<0.001$). Akita mice exhibited elevated blood glucose (WT-V 10.8 ± 0.6 mmol/L vs Akita-V 33.0 ± 0.0 mmol/L, $P<0.001$) and glycated haemoglobin (WT-V 4.60 ± 0.17 % vs Akita-V 13.1 ± 0.35 %, $P<0.001$). Total heart and individual ventricles (LV/TL: WT-V 6.18 ± 0.27 mg/mm vs Akita-V 4.50 ± 0.10 mg/mm, $P<0.01$) were all smaller in Akita mice. None of the aforementioned parameters were impacted by MitoGamide treatment (P=NS). Echocardiographic analysis confirmed that cardiac dimensions were smaller in Akita hearts (Ex-LVEDD: WT-V 5.75 ± 0.11 mm vs Akita-V 5.19 ± 0.05 mm, $P<0.01$). Diastolic dysfunction was evident in Akita mice, and notably, MitoGamide treatment preferentially improved several of these markers, including e'/a' ratio (Akita-V 0.81 ± 0.04 vs Akita-MG 1.25 ± 0.05, $P<0.001$) and E/e' ratio (Akita-V 31.39 ± 2.06 vs Akita-MG 24.89 ± 1.57, $P<0.05$).

Discussion: Our findings suggest that MitoGamide, a novel mitochondria-targeted approach, offers cardioprotection in experimental diabetes and therefore has therapeutic potential for the treatment of cardiomyopathy in patients with diabetes.

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Nitroxyl-based Therapies to Overcome Nitric Oxide Resistance in the Diabetic Heart and Vasculature

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Introduction: Nitroxyl (HNO) donors enhance cardiac contraction and relaxation, while promoting vasodilation in type 1 diabetes (T1D), whereas vascular and cardiac actions of nitric oxide (NO) are diminished, due to NO resistance (de novo impairment of tissue responsiveness to NO). However, the presence of NO resistance and the ability of HNO donors to overcome this in the heart and vasculature in type 2 diabetes (T2D) has not been examined.

Aims. To investigate if T2D promotes, and HNO circumvents, NO resistance in the heart and vasculature.

Methods: T2D was induced in male Sprague Dawley rats using a combination of a high fat diet (59% total energy from lipids) and streptozotocin (STZ, 2x35 mg/kg in citrate vehicle 0.1 M, ip). Control animals received vehicle and a standard chow diet. All rats underwent fortnightly blood glucose monitoring and glucose tolerance tests. Following 12 weeks of diabetes, vascular reactivity of second-order mesenteric arteries was assessed by wire myography. In addition, the heart was isolated to assess cardiac function while Langendorff-perfused.

Results: Blood glucose levels were elevated in diabetes (>20 mM) in comparison to control (~7 mM). Relaxation was impaired in diabetes in response to ACh (pEC_{50}, control: 7.98±0.19, diabetes: 7.28±0.17, $P<0.05$) and the NO donor diethylamine NONOate (DEA/NO; pEC_{50}, control: 7.46±0.28, diabetes: 6.88±0.15, $P=0.072$). However, relaxation in response to the HNO donor Angeli's salt was surprisingly enhanced in diabetes (pEC50, control: 6.66±0.23, diabetes: 7.54±0.16, P<0.05). Further, although myocardial contractility was lower in diabetes (Δ+dP/dt, control: 205±31, diabetes: 100±28 mmHg/sec, P<0.05), the response to Angeli's salt was enhanced (Δ+dP/dt, control: 158±20, diabetes: 352±68 mmHg/sec, P<0.05).

Discussion: In this model of T2D, both myocardial and systemic vascular responses to NO are impaired. However, HNO circumvents this NO resistance at both the myocardial and vascular levels. These effects highlight the therapeutic potential of HNO donors to treat cardiovascular complications in T2D.

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Post-stroke cognitive impairment is exacerbated in hypertensive mice

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Introduction: Cognitive impairment is an aging-related disorder that can arise as a result of cardiovascular pathology or cerebrovascular injury. Due to the aging of our population, the incidence of cognitive impairment is expected to rise. Hypertension is one of the major modifiable risk factors for stroke and dementia, but it is not known whether it may exacerbate stroke-induced cognitive impairment.

Aims. This study aimed to determine the effect of hypertension on post-stroke cognitive outcomes.

Methods: C57BL/6J mice (n=48) were randomly assigned to receive chronic infusion of either saline or angiotensin II (0.7mg/kg/day) via osmotic minipump. Seven days after minipump implantation, mice underwent either sham or stroke surgery. The photothrombotic model of ischemic stroke was used to target the prefrontal cortex, an area known to play an important role in working memory. At day 14, mice performed an object relocation task, designed to assess spatial recognition and working memory. Blood pressure was measured weekly by tail-cuff plethysmography.

Results: Angiotensin II increased systolic blood pressure (saline, 118±1 mmHg vs. Ang II 149±2 mmHg; P<0.05) but this was not affected by stroke (Ang II + sham, 151±4 mmHg vs. Ang II + stroke 148±2 mmHg). Normotensive mice that received sham surgery interacted with the relocated object approximately 60% of the time, indicating intact working memory. Hypertension or stroke alone did not significantly reduce the amount of interaction time with the relocated object. In contrast, the combination of hypertension and stroke resulted in mice interacting with the relocated object only approximately 35% of the time, indicating impaired working memory. Interestingly, we observed a high incidence of haemorrhagic transformation in the hypertension + stroke group, indicative of worsened injury.

Discussion: Our findings indicated that hypertension + stroke impaired working memory and worsened brain injury compared with either insult alone. Thus, controlling blood pressure is likely to be important for reducing the severity of cognitive impairment in stroke patients or in people at high risk of stroke.

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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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The role of prostaglandin E2 in mediating urinary bladder contractions

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Introduction: Current pharmacological treatments for overactive bladder (OAB) are not suitable for all patients, and there is interest in developing combination therapies that target different receptor systems involved in mediating spontaneous contractions in OAB. The release of inflammatory mediators, such as histamine (Stromberga et al, 2019) and prostaglandins may have a role in mediating bladder contractions.

Aims. This study to compare responses of PGE_2 on the urothelium with lamina propria (U&LP), or detrusor smooth muscle, and identify the receptor subtypes mediating PGE_2 contractile responses in these tissues.

PGE_2 conc.	Δ Tension (g)	Δ Frequency (cpm)	Δ Amplitude (g)	n
100 nM	0.33 ± 0.06	0.02 ± 0.17	-0.12 ± 0.04	4
1 μM	1.01 ± 0.08	1.13 ± 0.19	-0.14 ± 0.04	38
10 μM	1.46 ± 0.13	1.34 ± 0.57	-0.15 ± 0.06	18

Methods: In the presence of selective PGE_2 receptor antagonists, varying concentrations of PGE_2 were applied to isolated U&LP and detrusor urinary bladder strips mounted in organ baths filled with Krebs-bicarbonate solution and gassed with carbogen. Data analysis was performed using Student's *t*-tests. This research was supported by Australian Bladder Foundation.

Results: The addition of PGE_2 (1μM and 10μM) to U&LP preparations caused significant increases in baseline tension and spontaneous phasic contractile frequency (see Table). In detrusor preparations, significant increases in baseline tension were observed in response to PGE_2 (1μM and 10μM) and spontaneous phasic contractions were initiated in 83% of preparations. The antagonism of all four PGE_2 receptor subtypes revealed no changes in the contractile response observed after the treatment with PGE_2 in both U&LP and detrusor tissues. However, antagonism of EP1 receptor caused a significant delay in the time required to reach peak contractile response when compared to control tissues.

Discussion: PGE_2 elicits strong contractile responses in both U&LP and detrusor of porcine urinary bladder. This study presents the prostaglandin E_2 receptor system as having a potential role in mediating bladder contractile disorders, such as overactive bladder.

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

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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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Preliminary validation and application of a LCMS/MS method to quantify plasma efavirenz and metabolites in Papua New Guinea HIV/AIDS patients.

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Introduction: HIV/AIDS significantly impacts the health of the people of Papua New Guinea (PNG). The major drug therapy for HIV/AIDS in PNG is efavirenz (EFV) at a once-daily fixed dose of 600 mg (in combination with lamivudine and tenofovir or zidovudine). The unique genetics of PNG HIV/AIDS patients may place many of them at higher risk of major toxicities related to high plasma EFV concentrations (Tucci et al., 2018).

Aims. Develop a LCMS/MS method to quantify plasma EFV and metabolites (8-OH-EFV, 7-OH-EFV, 8,14-diOH-EFV) to support clinical pharmacology research in PNG HIV/AIDS patients.

Methods: EFV and metabolites were extracted from plasma (100 µL) by supported liquid extraction (Phenomenex Strata-DE) and 1 µL resolved on a C18 column (Phenomenex Kinetex 1.7 µm, 100 x 2.1 mmm, 40∞C) using 65:35 methanol:0.1% formic acid mobile phase at 0.4 mL/min (run time 4 min). Detection was by a LCMS8040 triple quadrupole MS (Shimadzu). Calibration curves ranged from 0.5-8 µg/mL (EFV, 8-OH-EFV) and 0.05-0.8 µg/mL (7-OH-EFV, 8,14-diOH-EFV). Preliminary assessment of inter-day (n=3) and intra-day (n=5-6) precision and accuracy was performed, and the method applied to trough plasma samples from 4 PNG HIV/AIDS patients (efavirenz 600 mg/day).

Results: Intra- and inter-day imprecision (CV) for quality control samples and lowest calibrator ranged from 1.9-10.1% and 0.4-5.4%, respectively. Mean intra- and inter-day accuracies ranged from 98-110% and 101-107%, respectively.

Plasma EFV and 8-OH-EFV concentrations in PNG patients ranged from 0.96 to > 8 µg/mL and 0.21-0.81 µg/mL, respectively, and 8-OH-EFV:EFV metabolic ratios ranged from <0.03 to 0.6. Plasma 7-OH-EFV and 8,14-diOH-EFV were detected in all samples, but concentrations were all less than 0.005 µg/mL.

Discussion: Preliminary findings demonstrate large interindividual variability in plasma EFV concentrations (>8-fold) and metabolic ratios (>20-fold) among PNG HIV/AIDS patients. However, further assay development and full validation areis required, especially for quantification of plasma 7-OH-EFV and 8,14-diOH-EFV.

Tucci et al. (2018) Pharmacogenomics 28(6):153-164

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Traditional Chinese herbs; the toxicity of polyherbacy in in vitro models of at-risk organ structures

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Introduction: Polypharmacy is growing issue in Western countries. In recent years, the popularity of complementary medicines, including herbal Traditional Chinese medicines, has increased. Due to the complex chemical nature of herbal products, there is an increased risk of unknown adverse reactions. As people from Western countries are more likely to use TCM preparations containing multiple herbs and multiple preparations, the likelihood of interactions is further increased. A recent case report saw a middle-aged female consume 12 raw traditional Chinese herbs resulting in wide-spread organ failure and death. The reaction was likely due to interactions between *Astragalus propinquus (A.pro), Atractylodes macrocephala (A.mac)*, each containing CYP3A4 inhibiting phytochemicals, and *Psoralea corylifolia* (*P.cor*), containing known hepatotoxic psoralen.

Aims. To investigate the impact of herbal combinations on herb-induced toxicity in models of at-risk organ structures.

Methods: *A.pro, A.mac* and *P.cor* were tested for individual and combined (0.1mg/ml of *P.cor* or *A.pro* in the presence of 0-5mg/ml A.mac) toxicity in kidney (BHK-21), intestinal epithelial (Caco2) and liver (HepG2) cell lines as models of at-risk organs. Cell viability was assessed using MTT colorimetric assays (n=4).

Results: A.*pro* showed significant toxicity at all concentrations in BHK cells ($p=0.0035$), 0.3mg/ml-5mg/ml in Caco2 cells ($p=0.0019$), and 1mg/ml-5mg/ml in HepG2 cells (0.0018). *A.mac* showed no toxicity in BHK cells but was significant at 3 mg/ml and 5 mg/ml in Caco2 and HepG2 cell lines ($p<0.005$). *P.cor* was significantly toxic in all cell lines; 0.1mg/ml-5mg/ml in BHK cells ($p<0.0001$), 0.3mg/ml-5mg/ml in Caco2 cells ($p<0.0001$), and 1mg/ml-5mg/ml in HepG2 cells ($p<0.0001$). *A.pro* in increasing *A.mac* showed no significant toxicity in BHK or Caco2 cells but was at 3mg/ml-5mg/ml in HepG2 cells ($p<0.0001$). P.cor with increasing A.mac showed significant toxicity at 5mg/ml in all cell lines ($p<0.05$), with significant interactions observed at 3mg/ml-5mg/ml in BHK cells. P.cor with increasing *A.pro* showed no significance in BHK cells, toxicity at 3mg/ml-5mg/ml in Caco2 cells and HepG2 cells ($p<0.05$), with a significant interaction at 5mg/ml in Caco2 cells.

Discussion: The results demonstrate that herbs have the potential for severe toxicity in key organ structures with the metabolism and interaction of these herbs potentially playing a significant role in the development of toxicity.

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Assessing quantum mechanical chemical descriptors for modelling toxicological outcomes

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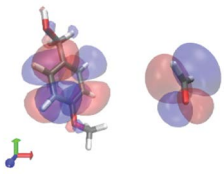

Introduction: The introduction of quantum mechanical electronic descriptors to characterise potential toxicants could improve the performance, reliability, and mechanistic interpretability of Quantitative Structure Activity Relationship (QSAR) models for predicting biological effects in computational toxicology. This could be implemented through improving 3D structure generation or the calculation of salient electronic features such as LUMO (pictured) energy values, which are a key determinant of Ames mutagenicity model performance (McCarren, *et al.* 2011). The Hartree Fock with 3 Corrections (HF-3c) methodology enables the large-scale calculation of electronic descriptors with a greatly reduced computation cost and increased robustness compared to previous low-cost quantum mechanical Methods:

Aims. This project aims to assess the QSAR performance of HF-3c-derived electronic descriptors and conventional descriptors augmented with quantum mechanical methodologies for modelling mutagenicity and Nrf2 assay outcomes.

Methods: A genetic algorithm automated model construction with 100 machine learning algorithms trained with 10-fold cross validation over 10 generations to optimise QSAR modelling performance using conventional Mordred descriptors with PM7 quantum mechanical or UFF forcefield generated 3D structures ($n=1825$) or HF-3c electronic ($n=21$) descriptors for predicting binary mutagenicity ($n=6512$), and numerical Nrf2 ($n=360$) outcomes.

Results: QSARs with PM7-enhanced conventional descriptors found better performance than QSARs using conventional descriptors in mutagenicity (0.915 vs 0.897 auROC) and Nrf2 (0.406 vs 0.423 MAE) while QSARs using HF-3c-derived electronic descriptors found similar performance in mutagenicity (0.872 auROC) and Nrf2 (0.420 MAE).

Discussion: The use of quantum mechanical methodologies in QSAR modelling methodologies presents mechanistic plausibility needed for use in regulatory toxicology contexts, enables robust modelling of small datasets ($n<1000$) without overfitting risks from using large descriptor sets ($n<1000$), and generally improves predictive performance.

McCarren P, Springer C, & Whitehead L (2011). Journal of Cheminformatics 3: 51. doi:10.1186/1758-2946-3-51

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Optimising the methylthiol reaction model for predicting in vitro Direct Peptide Reactivity Assay results

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Introduction: The Direct Peptide Reactivity Assay (DPRA) is routinely used to assess chemical skin sensitisation potential by quantifying the covalent interactions between the chemical and the cysteine residue of a seven-residue peptide. This mechanism is highly amenable to *in silico* modelling with contemporary quantum mechanical transition state searching methodologies for modelling the covalent interactions between a potential toxicant and a methylthiol molecule as a reduced model of the peptide (pictured). Previous implementations have low throughput, limited applicability to large molecules from omitting dispersion effects, and questionable reliability from the use of the B3LYP/6-31G* functional and basis set.

Aims. This project aims to refine the methylthiol reaction model implemented using the Growing String Methodology to improve computational cost, robustness for large molecules, and reliability for various DPRA reaction mechanisms.

Methods: The computational cost for a range of small and large basis sets, integral approximation methods, dispersion corrections and density functional approximations were evaluated for a small benchmark set of molecules (n=10). Configurations that fail to finish the benchmark set were removed from further testing in the validation set (n=128). The resulting activation energies were compared to *in vitro* DPRA results and in vivo LLNA and human Results:

Results: The addition of water catalysed the previously inert methylthiol-formaldehyde reaction (pictured). This reaction scaled non-linearly, at 56 minutes with 2 CPU cores to 37 minutes with 4 CPU cores. The RICOSX integral approximations reduced total computational time from days to under 3 hours for each configuration. The w97X functional with the D3 dispersion correction and Def2-TZVP basis set features an ideal compromise between computational cost and chemical accuracy. Full results will relate simulated activation energies to experimental outcomes.

Discussion: The optimisation of the methylthiol reaction model is a costly but important step to maximise the value derived from using quantum mechanical methodologies for simulating toxicological mechanisms totalling an order of magnitude reduction in runtime. Solvation may be a key factor in improving methylthiol reaction simulations.

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Hybrid toxicogenomic augmented structure activity relationships for chemical carcinogenicity prediction

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Introduction: Qualitative structure activity relationship (QSAR) models have shown success in detection of toxicological endpoints such as carcinogenicity particularly for genotoxic carcinogens. However, non-genotoxic carcinogens which act through a variety of less well characterised mechanisms and are more difficult to detect as there is a weaker connection between chemical structure and biological activity. This imposes limitations on their reliable use in the regulatory and drug development contexts. The augmentation of standard QSAR models with toxicogenomic features can better represent in vivo chemical carcinogenesis to modelling algorithms. This will expand the predictive range of current QSAR models and enable some mechanistic interpretability to a previously purely statistical analysis as toxicogenomic features may elucidate carcinogenic pathways.

Aims: This project aims to investigate and assess the performance of computational, chemical carcinogenicity predicting QSAR models upon the inclusion of toxicogenomic features.

Methods: Chemicals in Benigni's ISSCAN database (2008) (*n*=1133) were cross referenced for chemical gene associations in the Comparative Toxicogenomics Database (CTD, *n*=528). This produced 453 680 individual entries describing 34 879 unique affected genes. Gene features were refined utilizing CTD's curated gene-disease association; genes without cancer associations were removed. Toxicogenomic features (*n*=3715) were included as both binary perturbed and type of gene disturbance. These features were supplemented to physicochemical or quantum mechanical electronic descriptors and modelled with a battery of supervised machine learning algorithms tuned with a genetic algorithm implemented with TPOT.

Results: Early results with a small subset of manually selected gene data has shown that most algorithms produced a small increase in predictivity with toxicogenomic features in the descriptor set with further studies currently ongoing.

Discussion: Early results indicate that hybrid models show improvements on standard QSAR analysis with further analysis awaiting the completion of final experiments.

Benigni R, Bossa C, Richard AM, & Yang C (2008). A novel approach: chemical relational databases, and the role of the ISSCAN database on assessing chemical carcinogenicity. Ann Ist Super Sanita 44: 48-56.

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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.

Barber CM et al (2013) Toxicon 66:47-58
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.

[1] Brambilla et al. (2013) Basic Clin Pharmacol Toxicol 112:302-313; Brambilla et al. (2012) Mutagenesis 27:387-413.
[2] Bruschweiler and Merlot (2017) Regul Toxicol Pharmacol 88:214-226.

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The quality and potential toxicity of herbal medicines assessed via the two pronged approach of Metabolomics and Next Generation Sequencing.

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Introduction: Herbal medicine is increasingly popular due to public perceptions of safety. However, Adulteration and contamination of herbal and complementary medicines is a significant problem worldwide, yet the extent of the problem in Australia is unclear. We used a combination of Next Generation Sequencing, metabolomic and toxicological screening to evaluate contamination and adulteration in a range of herbal medicines available in Australia to gauge potential harms.

Methods: 243 processed herbal preparations in various forms and from various herbal medicine types (Western herbal medicines, Traditional Chinese Medicines and Ayurvedic preparations) were randomly selected and purchased from pharmacies, health food stores, traditional herbal retailers and online in Australian capital cities during 2014. Samples were processed for DNA analysis was through Next Generation Sequencing. Small-molecule analysis included liquid chromatography with quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) detection and heavy metal analysis using inductively coupled plasma with mass spectrometer (ICP-MS) detection.

Results & Discussion: 53% of the analgesic category herbal preparations were adulterated or contaminated. As were 40% of the psychotropic category and 56% of the weight loss and cardiovascular category. In many preparations the primary herbal ingredients could not be detected. In the psychotropic category 14% of the medicines were contaminated with heavy metals above the TGA advisory limit. Between 35-65% of medicines (depending on the category) had additional plant DNA. Between 6-31% of medicines (depending on the category) had animal DNA ranging from rat to bat. Between 5-14% of medicines (depending on the category) had pharmaceutical contaminants.

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Perhexiline limits both doxorubicin cardiotoxicity and cancer growth in a human osteosarcoma xenograft model

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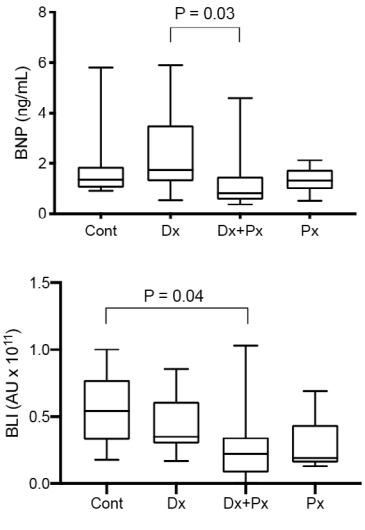
Introduction: Doxorubicin (Dx)-induced cardiotoxicity limits both duration and intensity of cancer chemotherapy. Oxidative/nitrosative stress and consequent activation of poly(ADP-ribose) polymerase-1 (PARP) underlie ATP depletion and cardiomyocyte damage. Perhexiline (Px) improves myocardial energetics and inhibits NOX2 activation (a major source of myocardial oxidative stress).

Aims. To determine whether Px can limit Dx cardiotoxicity without diminishing its anticancer effect.

Methods: Human osteosarcoma (BTK143) cells transfected with a luciferase tag were injected in the left tibia of nude (BALB/c) mice (9-12/group). After 7 days mice were treated with vehicle (Cont), Dx (4 mg/kg i.v. every 4 days), Px (200 mg/kg/day p.o.) or Dx + Px for a further 21 days. Cancer growth was assessed using bioluminescence imaging (BLI) and plasma B-type natriuretic protein (BNP) concentrations were measured as a marker of ventricular wall stress/myocardial inflammation. The effects of Dx (5 µM), Px (1 µM) and a PARP inhibitor (PJ34, 1 µM) were also investigated in 48 h human cardiomyocyte cell culture. Statistical significance was assessed by Kruskal Wallis or ANOVA.



Results: Dx + Px had the greatest anticancer efficacy, and also significantly decreased BNP concentrations compared to Dx alone (Figure). Px and PJ34 attenuated Dx cardiomyocyte toxicity in vitro, increasing cell viability from 62.5±2.3% (Dx) to 74.3±1.4% (Px + Dx, $P<0.05$) and 73.2±2.7% (PJ34 + Dx, $P<0.05$).

Discussion: Dx + Px was beneficial in vivo with respect to both cardiotoxicity and cancer growth, and also had a beneficial effect in vitro. However, our small sample size limits interpretation of the mechanism of this interaction.

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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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Are there any relationships between student demographics or their lecture attendance and academic outcomes?

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Introduction: In the modern digital age, it may be necessary to re-examine previously accepted dogma related to learning and teaching, such as lecture attendance being positively associated with academic outcomes.

Aims. For students with access to lecture recordings as part of the digital age, to determine if there are any relationships between student demographics or their lecture attendance and academic outcomes.

Methods: Students were studying biochemistry as part of a medical laboratory science degree in 2017/8. At the end of the unit, the relationship between demographics or lecture attendance and academic outcome was determined in an online survey. The academic outcome used was the overall final mark

Results: Forty-eight students were enrolled in 2017, 69 in 2018, and 71% and 84%, respectively, consented to undertake the survey. Many of the demographic factors were not associated with overall mark: gender (male/female), age when starting unit (under 21 or ≥ 21 years old), having a job (yes/no), or having a job for > 20 hours a week, English as first language or not, international vs domestic student. The one factor that showed a relationship was that students studying part-time had lower marks that those studying full-time. For lecture attendance (yes, most weeks; sometimes; no) there was no association between lecture attendance and the overall mark.

Discussion: The study suggests that students are good at regulating how much work and study they can do, with some students being able to combine high job loads with positive academic outcomes, and some of the weaker students having moved from full-time to part-time study to continue to achieve academic success. For the same cohort of students, when we used an attendance register at lectures, this also showed no association between lecture attendance and overall mark (Doggrell et al, 2018). Thus, in the modern digital age, it is no longer possible to generalize that lecture attendance is associated with better academic outcomes, as this was not the case for students studying biochemistry in a medical laboratory science course.

Doggrell SA et al. (2018). Proceedings of the Australian Conference on Science and Mathematics Education

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Designing and delivering a Biology Bridging unit to 1st year Pharmacy students

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Introduction: Biology is not a prerequisite subject for students enrolled in the Vertical Integrated Master's (VIM) degree in Pharmacy at Monash University. Approximately 60% of our student cohort have not studied Biology in Years 11/12 (62% in 2017; 60% in 2018; 55% in 2019). Without having done Year 11/12 biology, the transition burden of moving to higher education is greater given these students may also feel disadvantaged, overwhelmed and anxious about their lack of foundational biology knowledge, which may lead to an increase in poor educational outcomes.

Aim: To describe the process undertaken to design, develop and deliver a Biology Bridging unit to students enrolled in the VIM degree at the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University to bridge the knowledge gap in biology between secondary education and entry into Pharmacy.

Methods: We designed and developed two self-directed online biology modules (Module 1: Cells to Systems; Module 2: Organs and Organs Systems) to bridge the biology knowledge gap and introduce and/or refresh key biological concepts. Module 1 was designed as an introduction for students with no prior Biology knowledge; Module 2 was designed for students who had completed Module 1 or had studied Biology but wished to refresh and extend their knowledge. The online modules were delivered to students through the Learning Management System (Moodle) by an educational support team of assistant lecturers and designers.

Results: While our online modules were engaging (>90% of students accessed the modules in 2018-2019), students generally did not perform as well across the unit as their peers who had completed Biology. Of the bottom 10% performers in the unit, approximately 77% had not done Biology in VCE (83% in 2018; 64% in 2019). It was however interesting to note that the reduction in low performing students without a biology background, coincided with the offering of a revised biology bridging unit in the Pharmacy program in 2019.

Discussion: By introducing basic biological principles through our self-directed online modules, students were less anxious about their perceived lack of knowledge of Biology thereby improving confidence and enthusiasm. We hope that engaging with these resources will also enrich the experience of students with diverse knowledge-backgrounds. Feedback from the students will enable us to implement further changes to the modules in time for 2020.

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Development of scientific writing and referencing skills in pharmacology students

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Introduction: Inquiry, problem-solving and communication are important graduate attributes endorsed by the Australian Council of Deans of Science as some of the nationally agreed Biomedical Science Threshold Learning Outcomes (http://www.acds-tlcc.edu.au/science-threshold-learning-outcomes-tlos/science-tlos/). Students must also access, select and critically evaluate authoritative sources of information prior to communication.

Aims. To determine student perceptions and development of scientific writing and referencing skills.

Methods: Perceptions of scientific writing and referencing skills were examined in surveys administered to 2nd year pharmacology students at the start of semester 1 (survey 1, PHAR2210) and 2 (survey 2, PHAR2220, UWA Ethics Ref No. RA/4/20/5139). Survey responses were independently matched, coded and anonymised for analysis. Data are presented as mean + SD or percent respondents.

Results: Responses from 54 students who completed both surveys were analysed. Students were 19.6 + 2.2 years old, 57% were female, 93% domestic and 78% enrolled in a Pharmacology major. There was no significant change in perception between the two surveys, with over 94% of students Agreeing or Strongly Agreeing with the importance of developing written communication skills; over 96% developing scientific writing skills and over 78% developing reference skills. Over 11% of students held a neutral position on referencing skills. Survey 2 respondents had completed at least 1 scientific writing task at UWA, with most having completed between 1 and 5 (46%) or 6 and 10 (44%). Similarly, students had completed at least one task that included references, with most having completed between 1 and 5 (57%) or 6 and 10 (33%). The vast majority of written tasks required the use of references. While 80% of students reported that PHAR2210 had improved their scientific writing skills, fewer reported an improvement in referencing skills (69%). However, most students achieved high scores for referencing in PHAR2210.

Discussion: The vast majority of students recognised the importance of developing written communication, scientific writing and referencing skills and had completed at least one such task at UWA. That most students indicated an improvement in these skills in PHAR2210 was reassuring. That some students held a neutral position on referencing skills indicates that the importance of these skills could be better communicated to students.

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The IUPHAR Pharmacology Education Project: Opportunities and Challenges

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Introduction: The International Union of Basic and Clinical Pharmacology (IUPHAR) Pharmacology Education Project (PEP; https://www.pharmacologyeducation.org/) is an online learning resource. It contains open-source pharmacology content, reviewed by experts and available to support education and training in pharmacological sciences worldwide.

Aims. PEP aims to develop and curate freely available high quality contributions to pharmacology education. Such web-based materials are valued in particular by resource poor nations. PEP populates resources under the subsections: Pharmacology, Clinical Pharmacology, Drugs and Therapeutics.

Methods: PEP relies on the input from international pharmacology experts. We welcome contributions to any aspect of PEP that might be of value for learners. Currently, we call upon support of the community to develop the Drugs and Therapeutics sections. For more about the inception, management, format and goals of the Pharmacology Education Project see our Clinical Pharmacology and Therapeutics Practice article (1).

Results: Established in April 2016, PEP now receives approximately 16,000 visits a month, with over 400,000 page views since inception. The PEP also maintains accessory sites such as a Slideshare.net account which hosts slide sets submitted by educators. Based on the preliminary responses to an online survey, the information from PEP is used mostly in teaching and the quality is rated as excellent.

Discussion: One challenge of creating a large-scale open educational website of this kind is engaging educators and students in the building and maintenance of the site. The PEP has the potential to enhance significantly pharmacology knowledge across many disciplines worldwide. This will only come about, however, with assistance and contributions from the international pharmacology community.

References. 1) Faccenda, E. Maxwell, S. and Szarek, J. L. (2019), The IUPHAR Pharmacology Education Project. Clin. Pharmacol. Ther., 105: 45-48.

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Evaluating Multiple Choice Question (MCQ)-based summative assessments in Pharmacy Education: An integrative review

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Introduction: MCQ-based assessments are one of the most prevalent form of assessment utilized in Pharmacy Education programs, thanks to "its ability to test a broad scope of knowledge, its practicality, and ease of grading" (Pate et al, 2014). Despite the importance of MCQ-based summative assessments in Pharmacy Education curricula and the need for quality assurance, a holistic framework to evaluate the quality of MCQ-based summative assessments in Pharmacy education is still missing.

Aims. To decode elements constituting an evaluation of quality of MCQ assessments in Pharmacy and related Healthcare professional education emerging from research evidence from the literature.

Methods: An integrative review of ~1000 peer reviewed publications was conducted. Thematic analysis suggested four levels of evaluation: single test item, whole test, cross-unit assessment and cross-program assessment levels. Drawing on the concept of "validity" and "process of validation" (Cizek, 2012), each evaluation level included quality criteria, evidence for these criteria, methodology to collect, analyse and interpret data, and post-evaluation action plans.

Results: Findings from the integrative review highlights and explains the need for a holistic approach to evaluating MCQ-based assessments, which involves multiple levels of evaluation as well as multiple sources of evidence via the use of both quantitative and qualitative methodology.

Discussion: Suggestions from this review would inform the quality assurance, quality improvement processes, as well as designing professional development for academic staff in terms of assessment development and evaluation in pharmacy education and similar educational contexts.

Cizek, G. J. (2012). Defining and distinguishing validity: Interpretations of score meaning and justifications of test use. Psychological Methods, 17(1), 31.
Pate, A., & Caldwell, D. J. (2014). Effects of multiple-choice item-writing guideline utilization on item and student performance. Currents in Pharmacy Teaching and Learning, 6(1), 130-134.

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Medication reviews and geriatric medicine home visits

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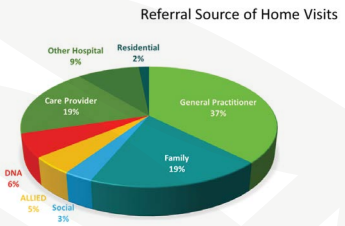
Introduction: Home visits by physicians in geriatric medicine provide an opportunity for medication reviews of older people in the community.



Aims. Review the home visits by medical team members of the ACAT (Aged Care Assessment Team) for medication reviews. Establish there are educational benefits for the patient and ACAT members.

Methods: An audit of home visits over a 6-month period. The medical records and community notes were reviewed for medication reviews, reason for home visits, source of referrals, purpose of the home visits and outcomes.

Results: There were 120 community home visits in 2018. In the last 6 months there were 65 home visits. 33 of the 65 patients had polypharmacy. 4 patients were on acetyl cholinesterase inhibitors for Alzheimer's disease but 19 of the patients at home had a dementia syndrome. 2 of the 65 patients were on benzodiazepine. The youngest client was 57 years old and the oldest was 95 years old. The average age was 81 years old. 24 of the patients were referred to a memory clinic for further medication review and therapeutic management.

Discussion: 50% of the community living patients had polypharmacy. There was a high risk for drug interactions. Poor compliance with medications could occur with nearly a third of the patients of having a dementia syndrome diagnosis. During the home visit the polypharmacy was brought to the attention of the accompanying allied health ACAT member, family members of the patient and carers. The general practitioner was provided with a written multi-disciplinary assessment that addressed the issues. The medical home visit provided an opportunity for educating all the stake holders in the patient's health care about the principles of quality use of medicines.

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Spaced education improves student performance in medical education

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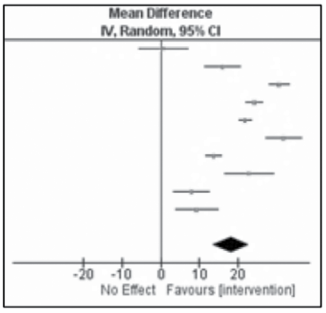
Introduction: Many services exist to support students enrolled in medical programs in Australia. The Australia Medical Council considers provision of student support under Standard 7 of its approved accreditation standards. While this standard considers professionalism, fitness to practice and student wellbeing, one thing that seems to be overlooked is training in study methodologies for assimilation of large volumes of information. Spaced education methods use temporally spaced challenges to reinforce retention of information by timing the challenges based on Ebbinghaus' forgetting curve (Murre & Dros, 2015).



Aims. The present work aims to evaluate spaced-education interventions implemented in medical schools in the hope that the method can be developed to help medical students at The University of Sydney.

Methods: A systematic review of the medical education literature and meta-analysis was performed.

Results: The results of the meta-analysis indicated that spaced-education programs improved final test scores although significant heterogeneity was found in the results (Fig.).

Discussion: Although significant heterogeneity was seen in the results this could be explained by the multitude of methodologies and settings included in studies where spaced-education has been implemented. Nonetheless the effect of the intervention was nearly always positive in the studies. Following on from these results an Education Innovation grant was prepared and a University wide implementation of spaced-education in pharmacology units was commenced in Semester one, 2019.

Murre JM Dros J (2015) PLOS ONE 10(7) :e0120644

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Conventional and Complementary Medicine Health Care Practitioners' perspectives on interprofessional communication: a qualitative rapid review

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Introduction: People have multi-faceted health care needs and consult a diverse range of health care practitioners (HCP) from both the conventional and complementary medicine healthcare sectors. Effective communication between HCP and with patients are obvious requisites to coordinating multidisciplinary care. Further, miscommunication is a leading cause of patient harm and is associated with reduced patient satisfaction, health literacy, treatment compliance and quality of life. In conventional healthcare settings, differences in professional hierarchy and communication styles are recognised communication barriers. Less is known about interprofessional communication (IPC) that includes traditional and complementary medicine HCP.

Aims. This review aims to summarise the experiences and perceptions of conventional and complementary HCP and identify factors that influence IPC.

Methods: A qualitative rapid literature review was conducted. Six databases were searched to identify original research and systematic reviews published since 2009 and in English. A thematic analysis of included studies was used to identify and explore important and recurring themes.

Results: A total of 18 articles were included, 11 of which reported on complementary HCP and seven were literature reviews. Four key themes were identified that impact IPC: medical dominance, clarity of HCP roles, a shared vision and education and training.

Discussion: IPC within and between conventional and complementary medicine HCP is impacted by interrelated factors. A diverse range of initiatives that facilitate interprofessional learning and collaboration are required to facilitate IPC and help overcome medical dominance and interprofessional cultural divides.

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Pharmaceutical Benefits Scheme (PBS) subsidised opioid medicines

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Introduction: A number of opioid medicines available in Australia are subsidised for acute and chronic pain conditions on the Pharmaceutical Benefits Scheme (PBS). There have been recent discussions to restrict pack sizes, particularly for schedule 8 opioid medicines ("Controlled Medicines"), however there has not been a comprehensive evaluation of the number of opioid medicines subsidised on the PBS or the cumulative opioid dose (in morphine milligram equivalents [MME]) in the subsidised medicine packs which could adequately inform decisions in this area.

Aims. To collect data on the number of opioid medicines subsidised on the Australian PBS, calculate the cumulative MME dose per subsidised packet and compare these with existing cumulative MME dose thresholds which are linked to increased risk of persistent opioid use at 1 year or beyond.

Methods: Two authors extracted data on all opioid medicines subsidised under the PBS in Australia. Using the Australian Medicines Handbook and the Centre for Disease Control opioid conversion charts, we determined the cumulative MME dose per packet and compared these with two cumulative MME dose thresholds (120 MME and 800 MME) where the risk of persistent use at 1 year is known to increase at least two-fold.

Results: A total of 92 opioid medicines were identified as being subsidised on the PBS for pain not explicitly related to cancer. Fifty seven of these are indicated for chronic severe disabling pain unresponsive to non-opioid analgesia. Thirty five were indicated for acute pain or pain of unspecified duration. In total, 95% of all subsidised opioid medicines had cumulative MME ≥ 120 and 45% had cumulative MME ≥ 800. Opioid medicines indicated for chronic pain were more likely to exceed these thresholds (98% and 58% respectively). Of all Schedule 8 opioid medicines subsidised under the PBS (n=82), 81 (99%) had cumulative MME ≥ 120 and 40 (49%) had cumulative MME ≥ 800.

Discussion: The vast majority of subsided opioid medicines exceed MME thresholds which have been linked to persistent opioid use at 1 year and beyond. This warrants careful discussion around strategies which can minimise these risks for patients commencing opioid analgesia for the treatment of moderate to severe non-cancer pain.

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Health consumers' perspectives of Saudi pharmacists' roles in cardiovascular disease prevention

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Introduction: Cardiovascular diseases (CVDs) can be caused by modifiable and non-modifiable risk factors. Identifying, assessing and managing modifiable risks at an early stage stands to limit CVD progression; community pharmacy presents an accessible venue for delivery of such risk management services. However, consumer perceptions about such pharmacy models have not been explored in Saudi Arabia.

Objectives. Therefore, this study aimed to explore the perceptions of health consumers about current and future CVD risk screening and management services by pharmacists in Saudi Arabia.

Methods: Semi-structured interviews conducted with consumers with at least one modifiable CVD risk factor. The interviews were audio-recorded, transcribed verbatim, translated into English and then thematically analyzed.

Results: A total of 25 health consumers, most of whom were Saudi (88%) and women (65%), participated in face to face interviews. Five main themes emerged from the analysis of consumers' responses. 1. *Perception of pharmacists' role:* which was mainly perceived to be medication supply. 2. *Trust and satisfaction with current service:* most participants indicated low trust/service satisfaction with pharmacists' care. 3. *Preferences for future pharmacy services:* there was willingness to engage in future pharmacy delivered CVD preventive health services, provided stringent regulation/ oversight of the quality of such services was assured. 4. *Viability of new pharmacy services:* public promotion, collaboration with other health professionals, financial incentivization and motivational rewards were thought of as essential ingredients to ensure service feasibility. 5. *Health beliefs and help seeking behaviors of consumers:* were diverse, low health literacy was evident and it was thought that pharmacists can help in these matters by educating and advocating for such consumers. Overall, the data suggested that clinical, communication and professional skills need to be enhanced among Saudi pharmacists to enable them to provide optimal patient centered services.

Conclusion. Health consumers participants were willing to participate and utilize CVD risk screening and management pharmacy-based services, when offered, provided their concerns are addressed.

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The effect of immunosuppressant prescribing patterns on patient outcomes in elderly Australasian kidney transplant recipients

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Background. Kidney transplantation is first-line treatment for patients with end-stage renal failure. There are currently no specific guidelines for immunosuppressant prescribing in elderly patients and little is known about the influence of drug choice on patient and graft survival.

Methods: Data were obtained from the Australia and New Zealand dialysis and transplant (ANZDATA) registry from 2000-2015. A survival analysis was conducted in elderly recipients (≥65 years of age) to identify associations between drug choice (tacrolimus vs cyclosporine, mycophenolate vs azathioprine) and number of prescribed medicines, on graft and patient outcomes at one year post-transplant. The analysis was performed on an adjusted and unadjusted death censored basis, using cox regression.

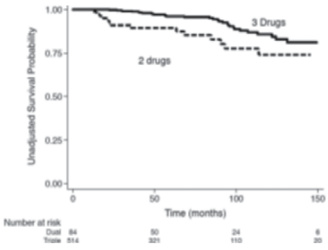

Figure 1 - Survival curve for dual versus triple immunosuppressant therapy in elderly recipients, adjusted for demographic and co-morbidity indices (p=0.0001)

Results: A total of 601 elderly patients were included in the analysis. Patients prescribed dual therapy compared to triple therapy, were more likely to have graft failure (95%CI 0.17-0.64, HR=0.33, p=0.001) (Figure 1). However this difference was not evident when censored for death (95%CI 0.12-1.28, HR=0.38, p=0.12). Time to graft failure was not found to be statistically associated with drug choice.

Discussion: Graft outcomes in the elderly appear to be largely unaffected by prescribing differences. Only patients prescribed dual immunosuppressant therapy were more likely to suffer graft loss. Findings from this study reinforce the value of current standard practice recommendations in Australia where patients are typically prescribed triple immunosuppressant therapy.

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Intensive rheumatoid arthritis therapy using a methotrexate loading dose schedule

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Introduction: Methotrexate (MTX) is the mainstay of rheumatoid arthritis (RA) treatment; however, it has large inter-patient variability in clinical response, 40% of RA patients do not response adequately and response is often delayed after starting treatment. Since long-chain RBC MTX polyglutamates (i.e. MTX-PG3, 4, 5) have particularly long half-lives, steady state concentrations are not achieved until weeks-months after treatment initiation. One strategy that could hasten the response to MTX is to administer it as a loading dose.

Aims. To investigate the pharmacokinetics of RBC MTX-PGs in patients given loading doses of MTX versus patients given the standard MTX dose.

Methods: Participants were randomized into two groups receiving either a 'standard' therapy including weekly dosing of MTX (10mg), or therapy that included 3x50mg MTX loading doses, after which MTX doses reverted to the standard 10mg per week. Doses were then modified according to clinical response and/or toxicity. Blood samples were collected at baseline then 1, 3 and 6 weeks after starting therapy. RBC MTX-PG concentrations were determined by tandem mass spectrometry, and linear mixed effects analysis was used to assess the relationship between MTX-PGs concentration and loading dose.

Results: There was significant higher concentration of MTX-PG4, MTX-PG5 and total MTX-PG concentration at week 1, 3 and 6 ($P < 0.05$). Long-chain MTX-PG were significantly higher in the 50mg group (45.4 and 68.9 nmol/L RBC at week 3 and 6 respectively), compared to the 10mg group (6.9 and 11.3 nmol/L RBC at week 3 and 6 respectively, $p<0.01$ for all comparisons), but there was no difference in the concentration of short chain MTX-PG.

Discussion: The concentrations of long-chain MTX-PGs were increased significantly in the loading dose group. This could decrease the time to achieve steady state concentration, increase intracellular retention of MTX-PGs and potentially hasten the onset of MTX response.

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Selection of suitable probe substrates to determine drug-metabolising enzyme activity in extracellular vesicles

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Introduction: Biomarkers from blood-based extracellular vesicles (EVs), such as cytochrome P450 (CYP) protein, mRNA, and *ex vivo* activity can be used to characterise variability in drug exposure. Previous reports have described lower CYP activity in plasma EVs compared to human liver microsomes (HLM) (Rowland et al, 2018). As such, the determination of *ex vivo* CYP activity in EVs requires the sensitive and selective analysis of *in vitro* probe substrates.

Aims. To identify suitable CYP3A4, 2D6, and 1A2 probe substrates for *ex vivo* metabolite formation assays in EVs.

Methods: EVs were isolated from the plasma or serum of healthy human volunteers (n=6) using ExoQuickTM Precipitation Solution (System Biosciences), membrane affinity, or size-exclusion chromatography. LC-MS/MS was used to quantify the formation of 1-hydroxymidazolam, dextrorphan, paraxanthine, and paracetamol from midazolam (CYP3A4), dextromethorphan (CYP2D6), caffeine (CYP1A2), and phenacetin (CYP1A2), respectively, in EVs and pooled HLM (n=5). Maximal rates of metabolite formation were used as ex vivo biomarkers for CYP activity in EVs.

Results: Metabolite formation was observed with all substrates in EVs except for phenacetin. Paracetamol formation was not observed in EVs due to minimal levels of contamination of phenacetin stock with paracetamol. Maximal rates of metabolite formation by EVs were 25.77 pmol.min-1mL plasma-1 plasma for 1-hydroxymidazolam, 10.12 pmol.min-1mL plasma-1 for dextrorphan, and 20.38 pmol.min-1mL plasma-1 for paraxanthine. Compared to HLM, maximal rates of metabolite formation were 100- to 1000-fold lower in EVs, presumably due to lower hepatic protein content in EVs.

Discussion: Midazolam, dextromethorphan, and caffeine demonstrated sufficient catalytic turnover, linearity of metabolite response, and an acceptable metabolite signal-to-noise ratio. However, phenacetin assay sensitivity was significantly compromised by stock contaminants, limiting its usefulness as a probe substrate. The effect of potential contaminants in other tested probes was negligible. These results indicate that midazolam, dextromethorphan, and caffeine are suitable probe substrates for *ex vivo* metabolite formation assays in EVs and highlight the importance of high substrate purity on assay sensitivity when working with EVs compared to HLM.

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Chronic polypharmacy and the Drug Burden Index (DBI) effects muscle function and structure in aged mice: association with gut microbiome

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Introduction: Ageing, polypharmacy (≥5 medications) and increasing DBI (anticholinergic and sedative medication exposure) are associated with impaired physical function. Preclinical ageing models can assess underlying mechanisms

Aims. We investigated whether chronic polypharmacy or monotherapy, with increasing DBI and/or cessation (deprescribing), affected physical function, fecal microbiome, and/or muscle histology in mice.

Methods: 12-month-old male C57BL/6 mice received either control diet or study drug(s) at therapeutic doses. Polypharmacy diets consisted of zero DBI (metoprolol, simvastatin, omeprazole, paracetamol, irbesartan), low DBI (metoprolol, simvastatin, omeprazole, paracetamol, citalopram) and high DBI (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin). Individual drugs (high DBI regimen) were tested as monotherapy. At 21-months, animals were randomised to continue treatment or gradually deprescribed. Rotarod testing and fecal collection occurred at 12-24-months, balance beam at 24-months, and Gastrocnemius muscle samples collected at 26-months.

Results: Citalopram mice (n=15-36; $p<0.05$) show reduced rotarod endurance for all time-points 15-months onwards, compared to control (n=24-29) and citalopram-deprescribed (n=12). Deprescribed high-DBI (n=18) and metoprolol (n=16) mice show better balance than prescribed comparators (n=10-15; $p<0.05$). 16S fecal microbiota sequencing shows increased Shannon-diversity ($p<0.05$) and reduced bacterial richness ($p<0.05$) for high DBI (n=23-12), compared to control (n=14-16) at 21-months, while deprescribing reduces Shannon-diversity to control levels. Preliminary histology results show reduced muscle collagen in high-DBI (n=6) compared to high-DBI deprescribed (n=6, $p<0.05$). Fibre type analysis in high-DBI (n=2), control (n=4) and simvastatin (n=3) mice indicate no difference between groups.

Discussion: Drug treatment affected fecal microbiota and rotarod, while deprescribing affected microbiome and balance. Preclinical results suggest polypharmacy and certain monotherapy regimens impact fecal microbiota, muscle function and may affect structure. Future research will finalise microbiome analysis and muscle histology data.

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NSW Cannabis Medicines Advisory Service (CMAS) methodology and evolution

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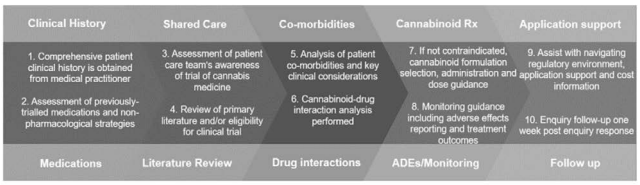

Background. The integration of cannabis medicines into clinical practice has occurred at an unprecedented speed. To support this, the NSW State government-funded CMAS has assisted clinicians to navigate this evolving translational research landscape since the Service's inception in January 2018. This abstract outlines NSW CMAS methodology in the provision of drug information in this novel area.

Methodology. NSW CMAS commonly receives patient specific enquiries related to patients with rare conditions, complex co-morbidities and extensive polypharmacy. NSW CMAS methodology in the context of a patient specific enquiry is outlined above. Results: Service evolution to date includes the receipt of over 1350+ enquiries, primarily from GPs (35%), other specialists (28%) and pharmacists (26%). The top three enquiry trends include patient specific enquiries, application support and product specific enquiries. Common patient specific enquiry types include chronic non-cancer pain, epilepsy, multiple sclerosis and spinal cord injury related spasticity and palliative care enquires.

Conclusion. NSW CMAS been extremely well received by clinicians, and has received both national and international attention. Service methodology is focused on supporting clinicians in understanding the latest evidence related to cannabis medicines and patient safety considerations. The evolution of the service highlights how a novel drug information service can be instrumental in the translation of an emerging field of research into clinical settings.

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Evaluation of fixed versus weight-based dosing regimens of intravenous vancomycin in patients receiving high-flux haemodialysis

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Introduction: Vancomycin is commonly prescribed in the high-flux haemodialysis (HFHD) setting. Many studies have explored fixed dosing (FD), yet few have explored weight-based dosing (WBD) regimens of vancomycin in this setting.

Aims. To evaluate FD and WBD regimens for vancomycin in HFHD in attaining pre-haemodialysis (pre-HD) concentrations of 15-20 mg/L.

Methods: This retrospective study evaluated 73 participants from two hospitals in Melbourne, Australia who received intravenous vancomycin across a five-year period. The FD regimens were classified as either a 1.0-1.5 g dose of vancomycin administered on- or off-dialysis. The WBD protocol was: 25-30 mg/kg loading dose and 20-25 mg/kg maintenance dose. Pre-HD vancomycin concentrations were evaluated at three consecutive HFHD sessions. Post-hoc population pharmacokinetic modelling was conducted and the 24-hour AUC was obtained for each individual vancomycin course. This was used to investigate the probability of attaining a 24-hour AUC/MIC ratio of ≥400.

Results: A total of 89 FD and 41 WBD courses of vancomycin were included in the analysis. The proportion of pre-HD vancomycin serum concentrations between 15-20 mg/L were 21.1-28.6% (FD group) and 18.5-41.2% (WBD group) across three consecutive HFHD sessions. The FD group had a greater proportion of pre-HD concentrations <15 mg/L, but a smaller proportion of pre-HD concentrations >25 mg/L than the WBD group. The probability of attaining a 24-hour AUC/MIC ratio of ≥400 over a 5-day period was <90% with FD and >90% on most days for WBD.

Discussion: The WBD regimen may be preferential to the FD regimen as fewer pre-HD concentrations were <15 mg/L. The probability of attaining a 24-hour AUC/MIC ratio of ≥400 was higher with WBD. Larger studies are warranted to further explore the impact of the two dosing strategies on clinical outcomes.

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Evaluation of data sharing statements within the top ten general and internal medical journals

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Introduction: As of the 1st of July 2018, the International Committee of Medical Journal Editors (ICMJE) mandated that submitted reports of clinical trials must contain a data sharing statement (DSS) outlining whether individual participant data (IPD) from the trial would be shared, when the data would be available, and where the data could be accessed. This study aimed to evaluate the first 6 months of DSS.

Methods: A structured search of PubMed was undertaken to identify registered clinical trials published between the 5th of July 2018 and the 2nd of January 2019 in the top ten general and internal medical journals by impact factor which included a DSS. The content of the DSS were evaluated.

Results: 133 publications of registered clinical trials were identified to have a DSS. Of the 133, 98 (74%) indicated IPD would be available, 22 (16%) indicated IPD would not be available, and 12 (10%) provided insufficient information to identify whether IPD would or would not be available for sharing. Of the 133 publications, 46 (35%) were from industry sponsored trials. Of these 46, 8 (17%) of the DSS indicated IPD would not be available for sharing. Of these 8 DSS, 2 (25%) were trials conducted by pharmaceutical companies with transparency policies to share IPD. Of the 46 industry sponsored publications, 34 (72%) had a DSS indicating IPD would be available for sharing. Of these 34 DSS, 23 (68%) gave a clear information on how to initiate a data sharing enquiry with the data holder, 2 (6%) indicated to contact the corresponding author of the industry sponsored trial, and 9 (26%) gave insufficient information to initiate a data sharing enquiry. New England Journal of Medicine (NEJM) manuscripts structured their DSS within a NEJM specific table; Journal of the American Medical Association (JAMA) and JAMA Internal Medicine manuscripts structured DSS under suggested subheadings; and the remaining sampled journals presented DSS as free text.

Discussion: Significant variability in the structure of DSS was identified between the journals. Sampled DSS indicated that a large proportion of trials (74%) would be available for IPD sharing at some point. Insufficient or conflicting information within the DSS was identified, which is not in alignment with the ICJME mandate.

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Population pharmacokinetic models of tacrolimus in adult transplant recipients: a systematic review

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Introduction: Numerous population pharmacokinetic (popPK) models of tacrolimus (TAC) in adult transplant recipients have been published to characterise the pharmacokinetic (PK) of TAC and facilitate dose individualisation.

Aims. To identify and compare published popPK models of TAC.

Methods: A systematic review was conducted using MEDLINE, EMBASE and the reference lists of all articles to identify

popPK models of TAC published up to 31 August 2019. Studies published in English and conducted in adults ≥ 18 years old were included.

Results: Of the 59 studies identified, 54% were conducted in kidney transplant recipients and 34% in liver transplant recipients. Only 2 studies were conducted in heart and 2 studies in lung transplant recipients. Most studies (90%) investigated the immediate-release formulation of TAC. The majority of studies (61%) used immunoassays to determine TAC concentration, followed by HPLC (27%) or a combination of both assays (12%). The number of subjects used to develop the models ranged from 12 to 681, with 60% of studies included less than 100 subjects. The median (range) concentration per subject used for model development was 14 (1–56). Model development was conducted using data from intensive sampling in 59% of the studies while the remainder used trough concentrations alone. Although the study period varied greatly (from immediate post-transplant up to a few years), most were performed in the first year following transplantation. Most models (80%) were developed using the NONMEM software. The PK of TAC was described using one- and two-compartment models, all with first-order elimination, in 58% and 39% of studies, respectively. Variability in TAC whole blood clearance amongst transplant recipients was most commonly related to CYP450 3A5 genotype, haematocrit and post-operative days. Variability in volume of distribution was mainly explained by body weight.

Discussion: Currently, little is known about the popPK models of TAC in heart and lung transplant recipients and for both the extended- and immediate-release formulations.

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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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Population pharmacodynamic modeling of sevoflurane as a bioequivalence test

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Introduction: Sevoflurane is a popular inhalation anaesthetic for general anaesthesia, and generic versions of sevoflurane have introduced from 2006.

Aims. This study aimed to assess the pharmacodynamic bioequivalence between generic and branded sevoflurane using nonlinear mixed-effect population pharmacodynamic (PD) modeling.

Methods: 128 patients undergoing elective surgery with general anaesthesia were allocated into either generic or branded sevoflurane in a randomized open-label fashion. Anaesthesia was induced by increasing the vapor setting of sevoflurane by 1 vol% up to 8 vol% via a facemask. The level of anaesthetic depth was measured by bispectral index (BIS), which is one of the processed electroencephalograms. The PD analysis using a sigmoid Emax model was performed to assess the relationship between the end-tidal concentrations of sevoflurane and BIS values (as surrogate measures of anaesthetic depth) using NONMEM® VII.

Parameter	Estimate (%RSE)	IIV variance (%RSE)
E_0	91.2 (0.37)	10.7 (13.2)
E_{max}	30.2 (4.70)	0.13 (41.4)
Ce_{50}	0.86 (13.3)	0.09 (25.1)
Gamma	4.35 (7.82)	0.47 (14.2)
Ke0	0.22 (21.0)	0.29 (31.9)

Results: During the sevoflurane induction with gradual increase of end-tidal sevoflurane concentration, BIS value was progressively decreased. The basic sigmoid Emax model adequately described the relationship between sevoflurane concentration and BIS value (Table). Various covariates were tested (e.g. age, body size), however, none were found to be significant for improving the model's ft to the data. The type of sevoflurane, as a categorical covariate, was also not a significant covariate for all PD parameters.

Discussion: PD parameters were not different according to the type of sevoflurane, generic or branded. The PD bioequivalence of inhalation anaesthetics might be proved by the PD modeling using a surrogate measure of anaesthetic depth, such as BIS.

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Prescribing practices of secondary stroke prevention medications at discharge from a large tertiary hospital

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Introduction: Stroke is the second leading cause of death and major cause of disability worldwide. Primary prevention has been successful in reducing the age-based incidence of stroke, however secondary prevention remains suboptimal. Studies indicate that 1 in 3 patients are not prescribed the recommended secondary stroke prevention medications at discharge from hospital.

Aims. To determine the current prescribing practices of secondary stroke prevention medications in patients post stroke or transient ischemic attack (TIA) at discharge from hospital.

Methods: A retrospective observational audit was conducted in a large tertiary hospital. Patients admitted under the stroke unit between August and September 2016 were identified for review. The primary outcomes were: (i) utilisation rates of the three key stroke prevention medications (antihypertensive, statin and antithrombotic agent) at discharge, unless contraindications exist; (ii) factors associated with the observed prescribing trends.

Results: A total of 111 medical records were reviewed, with 71 of these patients meeting the inclusion criteria (69.0% ischaemic stroke, 22.5% TIA and 8.5% haemorrhagic stroke). At discharge, 75.4% of eligible patients were prescribed an antihypertensive agent compared to 58.0% prior to admission ($P<0.05$). Forty-eight percent were discharged on a single antihypertensive agent, 36.5% on dual therapy and 15.4% on three or more antihypertensives. At discharge 66.7% of eligible patients were prescribed a statin compared to 35.0% prior to admission ($P<0.05$). At discharge 100% of eligible patients were prescribed an antithrombotic agent compared to 57.1% prior to admission ($P<0.05$). In total, 49.1% of eligible patients were discharged on all three key stroke preventative medications. Statistical analysis showed a number of factors affecting prescribing patterns including age, comorbidities and smoking status.

Discussion: This study successfully identified the current secondary stroke prevention prescribing patterns. With this information, strategies can be developed with the aim of improving medication utilisation.

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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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Unbound mycophenolic acid exposure is significantly higher prior to kidney transplantation, stabilising over the first 2 post-transplant weeks

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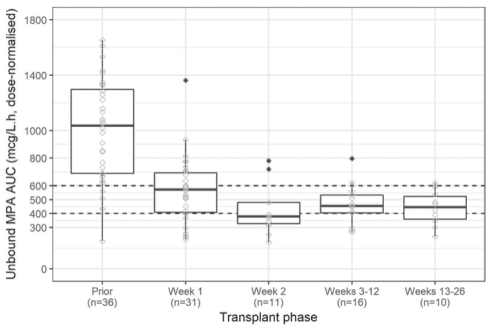
Introduction: Mycophenolate mofetil (MMF) dose optimisation has proven benefit in kidney transplantation, using a target concentration strategy. Total mycophenolic acid (MPA) PK changes over the initial post-transplant period, due to alterations in plasma protein binding.

Aims. To assess the change in unbound MPA exposure prior to transplantation and in the initial post-transplant weeks.

Methods: Adult and paediatric kidney transplant recipients had total and unbound MPA drug concentrations taken prior to transplantation (at steady state dosing) and at several post-transplant visits. Dose-normalised exposure (MPA AUC0-12) was calculated by linear-up log-down trapezoid AUC estimation. Exposure at different visits was compared using the paired Wilcoxon signed rank test and graphed using R version 3.4.3.



Results: Unbound MPA AUC0-12 was significantly higher before compared to after transplantation: median (IQR) 1073.6 mcg/L.h (723.1 – 1366.8 mcg/L.h), vs to 516.9 mcg/L.h (367.2 - 669.5 mcg/L.h), $p < 0.0001$. This fell further in week 2 (non-significant), without further evidence of systematic change in subsequent weeks.

Discussion: Dose optimisation to an unbound MPA exposure target, the 'effective' exposure metric, should reduce the need for frequent AUC estimation in the initial post-transplant months.

Metz D Holford NG et al (2019) Transplantation (In press)
de Winter B et al (2009) J Pharmacokinet Pharmacodyn. 36(6):541-64

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Validity and reliability of clinical resources on adverse effects of medicines: drug-induced liver injury

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Introduction: Patients need valid and reliable information about the adverse effects of medicines. Our service (www.mymedicines.nz) provides concise information for patients, focused on the most important points about the medicine. The adverse effects lists in most resources are overly long and inclusive, which patients find confusing and/or frightening. Our impression was that discrepancies between key resources are common, and that drug-attributable risk is poorly quantified. One of the most studied adverse effects is drug-induced liver injury (DILI).

Aim: To quantify inconsistencies and inaccuracies in the attribution of DILI in medicines information resources.

Methods: DILI attribution for 440 medicines in the MyMedicines database was extracted from four resources: two general resources (Micromedex Quick Answers® and the New Zealand Formulary); and two specialised adverse effect resources (Meyler's Side Effects of Drugs and LiverTox®). These were compared to medicines with DILI attribution determined by the Roussel Uclaf Causality Assessment Method (RUCAM).1 Data were analysed with descriptive and comparative statistics in SPSS™ v25.

Results: DILI was listed in at least one of the two general resources for 61% (268/440) of medicines and in at least one of the specialised resources for 42% (186/440). There was poor agreement between resources: Fleiss's kappa 0.32 (95% CI 0.28-0.36). Using RUCAM, DILI has been attributed to 6.6% (29/440) of the studied medicines. The sensitivity and specificity of the resources for DILI respectively were: 97% and 66% for Micromedex; 100% and 48% for the New Zealand Formulary; 93% and 61% for Meyler's Side Effects of Drugs; and 100% and 84% for LiverTox®.

Discussion: DILI risk is overstated in general resources and, to a lesser extent, in specialised resources. Over-emphasis on caution in these resources risks confusing clinicians and patients, and inconsistency between resources fails to support appropriate decision-making. Drug-attributable risk is often not quantified, and DILI is commonly described using non-specific terms (e.g. hepatotoxicity or liver damage). Inconsistency in adverse effect attribution in standard clinical resources has necessitated use of primary literature to develop valid patient information for MyMedicines.

1. Teschke R (2018) Expert Opin Drug Metab Toxicol 14:11, 1169-1187

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Development of an optimal sampling schedule for neonates and infants undergoing propofol infusions

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Introduction: Infusions of propofol are used for the maintenance of anaesthesia in neonates and infants. Drug delivery is controlled by infusion pumps that incorporate pharmacokinetic (PK) parameter sets. The parameter clearance (CL) is important to determine the target concentration of propofol. Differences in CL between adults and neonates and infants can be accounted for using body size and maturation. Normal fat mass (NFM), a size descriptor based on allometric theory and is fat free mass plus a component of fat mass, which is described using the parameter *Ffat.* *Ffat* is drug-specific and can be estimated for both CL and V.

Aim: To develop an optimal sampling schedule that provides accurate estimates of propofol CL and the drug-specific parameter F*fat* in neonates and infants undergoing propofol infusions.

Methods: A 3-compartment mammillary PK model with allometric scaling and a maturation component based on post-menstrual age (PMA) was used to describe propofol CL. Parameter estimates were sourced from pooled paediatric data1. The optimal design software PopED was used to optimise sample times in a population of 50 neonates and infants whose demographics were sampled from a local data base2. A maximum of six blood samples were allowed. Sampling windows were incorporated into the design to allow for deviations from the optimal sampling protocol.

Results: The sampled study population had PMA ranging from 44-137 weeks and weight from 2.9-15.0 kg. Optimal sample times (0, 1.2, 1.8, 38.6, 38.6 and 40.1 minutes) should estimate a relative standard error (RSE) for CL of 14%, 52% for *FfatCL* and 48% for *FfatV.* Efficiency of a 5 minute sampling window either side of the optimal time points was 85%.

Discussion: A sample size of 50 patients with this age and weight distribution appears too small to precisely estimate *Ffat.* Further investigation of different sampling designs to provide more precise estimates (RSE<20%) of these parameters is required.

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2. Sumpter AL, Holford NHG. Predicting weight using postmenstrual age–neonates to adults. Pediatric Anesthesia. 2011;21(3):309-315.

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Simultaneous quantification of four beta-Lactams in human plasma for therapeutic drug monitoring.

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Introduction: Therapeutic drug monitoring (TDM) has been proposed to optimise the dosing of beta-Lactam in critically ill patients as these patients might be challenging to dose due their pathophysiological changes. As no commercially available assay for TDM of beta-Lactams exists, an inhouse assay has to be developed.

Aims: To develop and validate an LC-MSMS assay for simultaneous quantification of the beta-Lactams piperacillin (PIP), tazobactam (TAZ), meropenem (MER) and ceftazidime (CFZ) in human plasma

Methods: Plasma samples were pre-treated with acetonitrile. Chromatographic separation of PIP, TAZ, MER, CFZ and their corresponding isotopically labelled internal standard were achieved using a C18 column and gradient elution of 90% acetonitrile. All compounds and internal standards were detected using electrospray ionisation in positive mode. Surplus patient plasma samples were used for incurred sample reanalysis.

Results: The total analysis time was 6.46 min. Calibration ranges was 0.05-100 mg/L for TAZ and MER, and 0.2-100 mg/L for PIP and CFZ (r2>0.998). Intra- and inter-day imprecisions were less than 15% and accuracy within 15% of nominal concentration in all cases. Variation between incurred patient samples for reanalysis were less than 12%.

Discussion: The developed assay provides a simple, sensitive and selective method for simultaneous quantification of PIP, TAZ, MER and CEF in human plasma for TDM. The concentrations observed from the incurred surplus patient samples were all within calibration range and were consistent with expected concentration given the timing of sampling and drug dosing. The lower limit of quantification level of 0.05 mg/L was crucial for the ability to quantify TAZ and MER within trough levels.

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

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Development of a novel LC-MS method to measure CYP2D6 activity in extracellular vesicles

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Introduction: Extracellular vesicle (EV) markers have been identified as a safe and non-invasive mechanism to account for between-subject variability in cytochrome P450 (CYP) 3A4, an important enzyme responsible for the metabolism of many clinically used drugs. Thus, EVs present significant potential in the field of precision medicine. Similarly, CYP2D6 displays high functional and expressional variability in the population resulting in sub-therapeutic or toxic effects. However, to date, there is no established method for the detection and measurement of CYP2D6 activity in EVs.

Aims. To develop and validate a LC-MS method for the measurement of *ex-vivo* CYP2D6 activity in EVs by using dextromethorphan (DXM) as a probe substrate.

Methods: Human serum samples were collected and EVs isolated by size-exclusion chromatography (qEV, Izon, New Zealand). Ex vivo incubations were performed in the presence of alamethicin-activated EVs, phosphate buffer, 25 µM DXM, and NADPH. Conversion of DXM to dextrorphan (DXO) was quantified by LC-MS. This assay was validated with respect to calibration linearity, recovery, matrix effect and variability.

Results: Incubation of EVs isolated from human serum with the CYP2D6 substrate DXM in the presence of NADPH for 2 hours resulted in the formation of approximately 2 nM of DXO. On this basis, a fit-for-purpose, high sensitivity ex vivo assay and LC-MS method was developed and validated to quantify DXO in human EV samples. Calibration curves were linear across a DXO concentration range of 0.5-5 nM. Comparison of calibration curves prepared in mobile phase and assay matrix demonstrated that recovery of DXO was >74%. Comparison of the response from spiked DXO into assay matrix from 5 participants demonstrated a lack of matrix effect on DXO quantification. Analytical variability (%CV) measured across 4 replicates at low and high DXO concentrations was 20.1 and 5.6%, respectively.

Discussion: A high sensitivity *ex vivo* assay and LC-MS method has been developed and validated to be fit-for-purpose to quantify between-subject variability in CYP2D6 phenotype. Application of this approach using serum derived EVs represents a novel and important advance in the capacity to account for differences in CYP2D6 activity due to either functional or expressional differences.

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Optimal design for the estimation of cefuroxime population parameters in the paediatric intensive care setting

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Introduction: Cefuroxime is a beta-lactam antibiotic administered for treatment of sepsis in infants and children. Despite common usage, PK parameter estimates in this population are few and none in the intensive care setting. Optimal design for PK studies can be used to identify designs that improve the precision of parameter estimates.

Aims: To develop an optimal sampling schedule for a population PK study in infants and children administered cefuroxime, in the intensive care setting.

Methods: A 2-compartment PK model was identified from the literature and parameter estimates were obtained from adult PK studies1,2. Initial estimates were allometrically scaled and included a maturation component. Demographic data for 50 infants and children were obtained by sampling from a patient database3. Renal Function (RF) was calculated using creatinine clearance and expected GFR based on post menstrual age (PMA) and weight. A maximum of 6 blood samples were assumed possible over an 8 hour period. The PopED optimal design package in R was used to obtain optimal sampling times using the D-optimality criterion.

CL	V1	Q	V2	TM_{50}	Hill
11 %	8 %	20 %	17 %	13 %	78 %

Results: Optimal sampling points in this population (44-768 PMA weeks, 2-85 kg weight, 0.29-2.08 RF value) were 0.01, 0.65, 0.76, 2.61, 2.78, 7.35 h after the first dose. Expected relative standard error values for each parameter in the optimal design are presented in the table. A 20 minute sampling window around each optimal sampling point had an efficiency of 96% compared to the discrete timed sampling optimal design.

Discussion: The optimal study design is suitable for estimating parameters with acceptable precision, except for the maturation Hill coefficient. A broader range of ages (e.g. neonates) may be required to estimate this parameter with improved precision.

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2. Rhodin, Malin M., et al. "Human renal function maturation: a quantitative description using weight and postmenstrual age." Pediatric nephrology 24.1 (2009): 67.
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Opioid exposure and cancer outcome

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Introduction: Opioids are widely used for the management of cancer pain, hence their ability to alter the course of cancer *in vitro* and *in vivo* has been of great interest to researchers and clinicians. Several cellular and animal models have demonstrated that opioids modulate tumour biology through direct effects on tumor growth and metastasis and indirect effects on immunity, inflammation and angiogenesis. However, due to difficulty in integrating evidence across multiple streams (*in vitro, in vivo,* and epidemiological data) it is unclear whether exposure to opioids promotes the occurrence of tumours in a cancer-free population.

Aim: Develop methods that allow the integration of different streams of evidence (cellular and animal research, evidence in humans and populations) to assess the effect of opioids on cancer outcomes.

Methods: We combined systematic review methods with an explicit causal framework for assessing biomedical evidence. We sought to identify and evaluate evidence of key mechanisms linking opioid exposure and cancer and then assess the available outcome data in light of these mechanisms. Key steps 1) identify and evaluate tumor-modulating mechanisms within the biological literature, 2) employ systematic review methods to assess cancer outcome data in different populations exposed to opioids, and 3) amalgamate the biological mechanistic data, plausible mechanisms that may confound population data, and the population data to assess the causal claim that opioids effect cancer outcomes in humans.

Results/Discussion: The assessment of whether opioid exposure influences cancer outcomes in a cancer-free population tends to focus either on findings from experimental biological sciences, or epidemiological data. The proposed method provides a principled way to amalgamate this evidence. The expected outcome of this approach is to be able to identify if there is an effect of opioids on cancer outcomes in initially cancer-free patients and to better define areas of remaining uncertainty.

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Accuracy of documented administration times for intravenous antimicrobial drugs

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Introduction: Therapeutic drug monitoring (TDM) is recommended for many antimicrobial drugs and assumes an accurate documented time of drug administration. Anecdotal evidence suggests that this is not the case.

Aims. (1) To determine the discrepancy between actual and documented administration times for monitored antimicrobial drugs. (2) To determine whether this discrepancy is impacted by (a) the day of the week, (b) the time of day, and (c) nurse-to-patient ratio. (3) Identify other medication administration errors which may influence the interpretation of drug concentrations and thus drug dosing decisions.

Methods: Patients receiving intravenous antimicrobial therapy were identified from the electronic prescribing system. Relevant patient data (e.g. ward location) and drug dosing data (e.g. medication, documented time of administration) were collected from medical records. Time and event logs were downloaded from the infusion pumps to determine the actual infusion start time. The influence of the day of the week, time of the day (based on nursing shifts) and nurse-to-patient ratio (intensive/critical care versus general ward) on the discrepancy of the administration time was examined.

Results: Of the 660 infusions (for ten different drugs), the median discrepancy between the actual and documented administration time was a 16-minute delay (range, 2-293 min) with discrepancies of more than 60 min occurring in 7.7% of administrations. Overall, discrepancies (median [range]) were similar on weekends (17 [2-293] min) and weekdays (16 [2-188] min) and high and low nurse-to-patient ratios (16 [2-157] min vs. 16 [2-293] min, respectively). Further, discrepancies were smaller for night (14 [3-91] min) compared to the evening (17 [3-293] min) and day (17 [2-248] min) administrations. Additional errors identified included one-third of administrations not being flushed through infusion pumps, numerous programming errors and prioritisation of particular medications over others.

Discussion: In general, the accuracy of recorded intravenous antimicrobial administration times was reasonable. Numerous, potentially clinically significant, medication administration errors were identified. Further investigation into these errors, their potential impact on drug dosing decisions, and workflow issues contributing to them is warranted.

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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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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.

Aims. 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 sEVs 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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Diabetic complications prevalence among patients treated with oral hypoglycemic agents and/or insulin at Suva Diabetic Hub from 2015 – 2017

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Introduction: Diabetes prevalence in Pacific island countries are among the highest globally and in Fiji, an estimate of 57, 640 type 2 diabetes cases have been reported with expectations for a rise annually. Likewise, diabetic complications rates are expected to rise, however to date the prevalence of complications in the country is scarcely reported.

Aims. The aim of this research was to identify common diabetic complications, its prevalence, associated therapeutic profiles and risk factors among type 2 diabetic patients seen at the National Diabetes Centre, Suva from 2015 to 2017.

Methods: A retrospective cross-sectional audit of type 2 diabetic patient folders attending the National Diabetes Centre was carried out (n = 355). Data collated included patient demographics, physical and biochemical parameters that were analyzed for statistical significance using the Chi-squared and one-way ANOVA tests.

Results: The average duration of diabetes prior complications development was 8 year with the average age at diagnosis being 44 years. Common diabetic complications in descending order of prevalence were nephropathy, foot ulcers, retinopathy, peripheral neuropathy and foot amputations with the associated risk factors being hypertension, overweight/obesity and poor glycemic control. 31.8% of patients were taking oral hypoglycemic agents only, 32.4% of patients were taking insulin only and 35.8% of patients were taking both oral hypoglycemic agents and insulin prior to the development of complications. There were no significant associations between the number of complications developed and the type(s) of pharmacological treatment received.

Discussion: The results highlights the need for a novel approach to better glycemic control among diagnosed patients and curtail the prevalence of diabetic complications. A large scale study would be best conducted to better understand the effectiveness of anti-diabetic therapy nationwide.

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Barriers and facilitators to user acceptance of a pilot therapeutic drug monitoring advisory service for vancomycin

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Introduction: Therapeutic drug monitoring (TDM) is recommended to optimise vancomycin therapy. Although literature demonstrates that vancomycin TDM can be enhanced by interventions, such as dose prediction software, there is a paucity of knowledge to support successful translation and sustained uptake of interventions in hospitals. In July 2018, a pilot vancomycin intervention, the TDM Advisory Service, was introduced at St. Vincent's Hospital, Sydney (SVH).

Aim: To identify the barriers and facilitators to acceptance of dose advice from the TDM Advisory Service by health professionals (prescribers and pharmacists) at SVH.

Methods: Health professionals (n=22) from a range of clinical units at SVH participated in semi-structured interviews. Broadly, interviews elicited information on knowledge of the TDM Advisory Service and factors influencing acceptance of dose advice. Interviews were transcribed verbatim and analysed independently by two researchers for emerging themes. The Theoretical Domains Framework (TDF) was used to synthesise outcomes relating to barriers and facilitators to uptake of dose advice.

Results: The main barriers to acceptance of dose advice aligned with three TDF domains; 'Environmental Context and Resources' (poor communication between the Service and its end-users), 'Social/Professional Role and Identity' (prescribing hierarchy, professional autonomy) and 'Knowledge' (uncertainty of Service capability). The main facilitators aligned with two TDF domains; 'Environmental Context and Resources' (electronic communication) and 'Beliefs about Consequences' (potential for improved patient outcomes).

Discussion: The TDF aided in categorising and prioritising key barriers and facilitators influencing acceptance of dose advice from the TDM Advisory Service by health professionals. The next phase of this work will be to map evidence-based behaviour change techniques to key TDF barriers and facilitators identified, which can then be operationalised into context-specific intervention strategies to support implementation of the TDM Advisory Service.

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Single-centre retrospective audit on the use of tapentadol and discharge communication

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Characteristic	n= 106
Age (yrs)	49.2 (+/-16.9)
Gender (Male)	51 (48%)
Surgical Admissions	88 (83%)
Median Length of Stay (days)	6 (IQR 3 – 10)
Initiated on Tapentadol	53 (50%)
Adverse Drug Reaction to Tapentadol	4 (3.8%)
Adequate Discharge Communication	53 (50%)

Introduction: Tapentadol is an atypical analgesic with μ-opioid receptor agonist and noradrenaline reuptake inhibitor activity approved for chronic severe disabling pain not responsive to non-narcotic analgesics. There is limited post-marketing surveillance data on usage or monitoring of efficacy and safety.

Aims. To review patterns of inpatient use and discharge communication and compare characteristics of patients initiated on tapentadol in hospital and those continuing therapy as a pre-hospital medication for pharmacovigilance.

Methods: Retrospective audit of all inpatients >18 years prescribed and dispensed tapentadol between January 2019 and March 2019 at a major tertiary centre.

Results: 106 patients received tapentadol during their hospital admission, with this being commenced in hospital for 53 patients (50%). 28/53 patients (53%) were subsequently discharged on tapentadol for ongoing analgesia and 17/28 (61%) of those met Pharmaceutical Benefits Scheme criteria. 4 patients (3.8%) were identified as having an adverse reaction to tapentadol during their admission. Major drug interactions with serotonergic drugs were identified in most patients, including when tapentadol was initiated in hospital and when it was continued as a pre-existing medication (64% vs. 76%, p=0.204). Discharge communication regarding analgesic changes was adequate in 53 patients (50%) administered tapentadol with no difference between those initiated or continuing regular tapentadol (p=0.560). This was despite frequent documented recommendations from the specialist pain service for those commenced on tapentadol in hospital compared to those continuing tapentadol from prior to admission (81% vs 32%, p<0.001).

Discussion: This audit has highlighted concerns around potential inappropriate prescribing in acute pain, recognizing drug interactions and discharge communication, which will be the focus of our targeted analgesia stewardship service.

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Monitoring for phenytoin toxicity in patients with hypoalbuminemia

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Introduction: Phenytoin is highly protein-bound (>90%) with 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 in hypoalbuminemia may not be recognised when only total concentrations are monitored. The Sheiner-Tozer equation accounts for this and has been validated against measured free concentrations, 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 phenytoin levels.

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

Results: Phenytoin concentrations were monitored for 144 patients with a total of 297 tests performed between January-December 2018. Moderate-severe hypoalbuminemia (<30 g/L) was noted in 47 (32.6%) patients and 48 (33.3%) patients were aged >65 years. Measured total phenytoin trough concentrations were undetectable for 27 (18.8%) patients. Supratherapeutic (>20mg/L) total concentrations were recorded for 12 patients (8.3%), which increased to 29 (20.1%) when adjusted for albumin (p=0.008). Significant neurological adverse effects were documented in 85 patients (59%), but this rarely led to a change in management despite supratherapeutic levels.

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.

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The role of UGT enzymes in cytotoxic drug resistance in breast cancer

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Introduction: UDP-glycosyltransferases (UGTs) are a family of drug metabolising enzymes that facilitate inactivation of xenobiotics, including therapeutic drugs, by conjugating them with sugars (e.g. glucuronic acid). Glucuronidation occurs in the liver to promote systemic detoxification/clearance, and in multiple drug-target tissues resulting in local inactivation. Epirubicin (EPI) is a cytotoxic drug used in combination therapies for breast and other cancers. UGT2B7 is the only UGT known to metabolize EPI. We previously showed that EPI induces UGT2B7 in liver cells, which may enhance systemic clearance. However, the role of UGT2B7 in intratumoural inactivation has not been examined.

Aims. To assess both the regulation and function of UGT2B7 in various breast cancer subtypes in the context of EPI treatment and to understand whether UGT2B7 has a role in drug resistance.

Methods: Realtime-PCR quantification was performed in breast cancer cell lines ZR-75-1 (p53 wildtype) and MDA-MB-231 (p53 R280K missense) to determine whether UGT2B7 expression is increased by EPI treatment. Promoter-reporter assays were used to characterize UGT2B7 induction via p53-dependent and -independent mechanisms. Stable UGT2B7-overexpressing cell lines were generated and characterized for response/resistance to EPI.

Results: 24-hour treatment of ZR-75 cells with 0.5 µM EPI resulted in ~20-fold induction of UGT2B7, whilst treatment of MDA-MB-231 cells with 1 µM EPI induced UGT2B7 ~7-fold (n= 3, P<0.02). The proximal UGT2B7 promoter region was induced ~130-fold (n=3, P<0.09) by EPI in ZR-75 cells; deletion of the p53 site within this region essentially abolished the activation. In contrast, EPI had negligible effect on UGT2B7 proximal promoter activity in MDA-MB-231 cells. These data suggest that EPI regulates UGT2B7 by p53-dependent and also -independent mechanisms in breast cancer cells. Overexpression of UGT2B7 in MDA-MB-231 increased the IC50 for EPI almost 2-fold (n=3, P<0.005).

Discussion: EPI induces UGT2B7 in breast cancer cells. In p53 WT cancers this effect is largely mediated by a proximal promoter p53 site; however, p53-mutant cancers may also induce UGT2B7 by a p53-independent mechanism. Proof of principle that elevation of UGT2B7 can reduce EPI sensitivity suggests that the induction of UGT2B7 by EPI could contribute to resistance to EPI-containing therapies. Further defining these feedback pathways may provide new avenues to enhance the efficacy of cytotoxics by modulating UGT2B7 activity.

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Impact of phenotype and pregnancy related induction of CYP2D6 expression on exposure to codeine and morphine following oral codeine dosing

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Introduction: Cytochrome P450 (CYP) 2D6 is a polymorphic drug metabolising enzyme that exhibits marked between subject variability in activity. An individuals CYP2D6 phenotype is broadly classified as extensive (EM), poor (PM) or ultra-rapid (UM) based on genotype driven differences in in protein expression. While access has become more restricted in recent years, codeine remains a frequently used mild analgesic. While CYP2D6 is not susceptible to traditional (pregnane X receptor mediated) induction, recent repots have demonstrated marked induction in the third trimester of pregnancy. The therapeutic effect of codeine, and risk of toxicity, is impacted by the CYP2D6 mediated demethylation of this drug to morphine.

Aims. Use physiologically based pharmacokinetic (PBPK) modelling to evaluate the impact of CYP2D6 phenotype and pregnancy status on exposure to codeine and morphine.

Methods: PBPK models for codeine (substrate) and morphine (metabolite) were developed and verified using Simcyp (v17.1). Population profiles for EM, PM and UM phenotype non-pregnant, pregnant and 3rd trimester pregnant females were developed by adjusting CYP2D6 abundance and genotype frequency values in the inbuilt healthy volunteer and pregnancy population files.

Results: Codeine exposure was modestly effected by CYP2D6 phenotype and pregnancy status. Substantial differences in morphine exposure were observed between CYP2D6 phenotypes and pregnancy status; morphine AUC in 3rd trimester pregnant EM and UM females (367 and 597 ng.mL.hr-1) were markedly higher than pregnant EM and UM females (220 and 368 ng.mL.hr-1), or non-pregnant females (201 and 348 ng.mL.hr-1). PM CYP2D6 phenotype did not form morphine, irrespective of pregnancy status.

Discussion: Third trimester pregnancy status and UM CYP2D6 phenotype were associated with marked increased in morphine exposure. The AUC for 3rd trimester UM CYP2D6 phenotype females (597 ng.mL.hr-1) was three fold higher than non-pregnant EM CYP2D6 phenotype females (201 ng.mL.hr-1). The marked increase in morphine exposure following codeine administration has the Third trimester UM CYP2D6 pregnant females increases risk of foetal respiratory depression.

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Use of PBPK modelling to identify covariates driving variability in sunitinib exposure and predict optimal dosing

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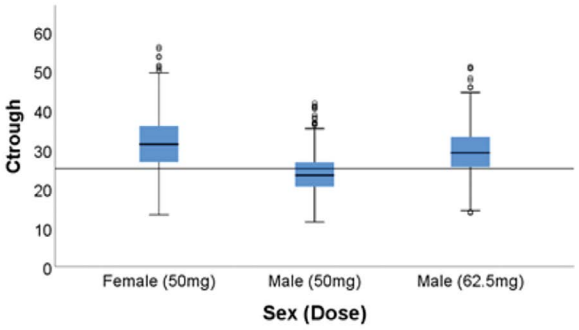
Introduction: Sunitinib is a vascular endothelial growth factor receptor inhibitor indicated for the treatment of advanced renal cell carcinoma, gastrointestinal stromal tumour after failure of imatinib mesylate treatment and of unresectable, well differentiated neuroendocrine tumours. There is high inter-patient variabilities in exposure to sunitinib yet all patients are started on the standard schedule of oral 50mg daily for 4 weeks followed by a 2 week rest period. There is a high rate of dose reduction and discontinuations due to adverse effects from this schedule, it has also been theorised that many patients may not be receiving adequate treatment.



Aims. To identify physiological characteristics in the cancer population that drive variability in sunitinib exposure using physiologically based pharmacokinetic modelling.

Methods: A full PBPK model incorporating mechanistic absorption was developed and verified for sunitinib using Simcyp (Version 17.1) in accordance with the US Food and Drug Administration Guidance for physiologically based pharmacokinetic analyses. This model was used to simulate sunitinib exposure over 14 days with daily dosing (50 mg) in a cohort of 2,000 cancer patients.

Results: The main physiological drivers of variability in sunitinib exposure were sex (ROCAUC .743), weight (ROCAUC .834) and height (ROCAUC .851). A multivariable linear regression based on these parameters predicted sunitinib Ctrough with an R2 of .665. Importantly, strong predictive performance in terms of identifying individuals likely to fail to achieve a therapeutic sunitinib concentration was demonstrated for a multivariable logistic regression model accounting for these variables (ROCAUC .907).

Discussion: Gender was identified as a significant variable as it can have the greatest clinical impact. A step towards a more personalised medical approach is being aware that on average, males tend to be subtherapeutic and will have the greatest benefit with early dose adjustment or a higher starting dose (62.5 mg).

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A clinical study to assess the magnitude, time-course and variability of Human Cytochrome P450 3A4 induction by rifampicin in a healthy volunteer cohort

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Introduction: Cytochrome P450 (CYP) 3A4 is the drug metabolising enzyme of greatest clinical importance. Consistent with its important role, CYP3A4 activity is a central determinant of drug exposure; metabolic drug-drug interactions (DDIs) that induce CYP3A4 activity are an important source of variability in exposure for drugs metabolised by this enzyme. Despite the large propensity for metabolic DDIs that induce CYP3A4 activity, there is no prescriptive dosing guidance for clinical DDI studies that use the established CYP3A4 inducer rifampicin to assess the clinical DDI risk of drugs metabolised by CYP3A4. Recent simulation data indicate that dosing rifampicin for less than 10 days is likely to underestimate the magnitude of induction.

Aims. Track the time course of CYP3A4 induction following administration of rifampicin over 15 days in a cohort of healthy males and females. The impact of rifampicin dose was assessed as a trial characteristic effecting the magnitude of CYP3A4 induction.

Methods: Healthy volunteers were dosed with midazolam (1 mg PO on Day 1, 8, 15) and timed blood samples (8mL) were collected pre-dose, and up to 6 hr post-dose. Participants self-administered a 14-day course of rifampicin (300 or 600 mg PO daily). Midazolam exposure was measured prior the first rifampicin dose (Day 1), following seven doses of rifampicin (Day 8) and 16 hours after completing the 14-day course rifampicin (Day 15). Baseline CYP3A4 activity was compared with activity following administration of rifampicin for 7 and 14 days.

Results: The mean midazolam AUC ratio following dosing of rifampicin (300 mg) for 7 days was 3.16, the mean midazolam AUC ratio following dosing of rifampicin (600 mg) for 7 days was 3.92. No significant difference in AUC ratio was observed between analysis performed after 7 and 14 days rifampicin dosing (600 mg).

Discussion: Consistent with prior studies. 25% greater CYP3A4 induction was achieved using 600 mg rifampicin. While prior analysis has shown that at least 10 days rifampicin dosing is required to achieve maximal induction of hepatic CYP3A4, these data indicate that 7 days dosing is sufficient to demonstrate maximal change in midazolam exposure.

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Serum-derived small extracellular vesicles as diagnostic biomarkers for non-alcoholic fatty liver disease.

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Introduction: Non-alcoholic fatty liver (NAFL) is a highly prevalent chronic liver disease with variable severity, from simple steatosis to steatohepatitis (NASH). NASH involves hepatocyte death, inflammatory infiltration and fibrosis, with the potential to progress to cirrhosis and hepatocellular carcinoma. Given the prevalence and spectrum of severity, this disease is a source of marked variability in the function of drug metabolism pathways, and hence drug exposure. Liver biopsy is currently the only way to accurately diagnose NASH but is highly invasive and poses risks to patients. Circulating populations of small extracellular vesicles (sEVs), including exosomes (30-150nm) and microvesicles (50-1000nm), are known to exhibit alterations in concentration and molecular cargo in disease states, including NAFL and NASH. Thus, they may be used as a "liquid biopsy" to diagnose and stage disease severity in a simple and minimally invasive manner.

Aims. This study aimed to determine if differences in abundance, size and molecular cargo of serum-derived sEVs could differentiate patients with NAFL or NASH from healthy individuals.

Methods: Serum samples were obtained from healthy volunteers (n=17) and patients with NAFL (n=5) or NASH (n=12). sEVs were isolated by ExoQuick precipitation solution and the abundance and size distribution analysed by nanoparticle tracking analysis (NTA). RNA was extracted using TRIzol reagent and reverse transcribed using TaqMan miRNA RT kit. Expression of miR-122, -451, -16, -192 was determined by RT-qPCR using TaqMan small RNA assays.

Results: Abundance of circulating sEVs was higher in individuals with NAFL than either healthy individuals ($p=0.035$) or individuals with NASH ($p=0.005$). sEVs were larger in both NAFL and NASH patients compared to healthy ($p<0.001$) and differences in purity suggested a greater microvesicle population in diseased serum. Expression of liver miRNAs (miR -122 and -192) increased relative to ubiquitous EV miRNA (miR -451 and -16) with mean fold-changes of 6.7 in NAFL and 5.2 in NASH. miR-122 expression relative to miR-192 also increased 3-fold in NAFL and 1.9-fold in NASH.

Discussion: These data indicate that sEV abundance cannot delineate healthy and NASH subjects. With respect to EV-derived miRNA in NAFL and NASH, there is a proportional increase in liver-derived miRNA. The increase in miR-122 relative to -192 may be reflective of disease-associated changes in liver-specific EV miRNA expression.

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Isothiocyanates from Cruciferous Vegetables and Breast Cancer: A Systematic Review

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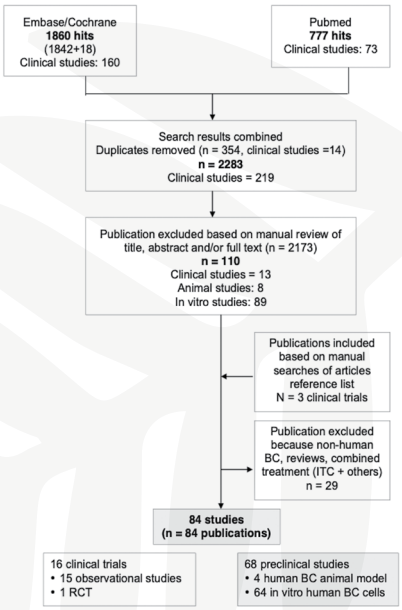
Introduction: The effect of cruciferous vegetable intake on breast cancer survival is controversial.

Aims. This study aims to systematically review the evidence from all types of studies, which examined the effect of cruciferous vegetables or their isothiocyanate (ITC) constituents on human breast cancer.

Methods: A systematic review was conducted in Pubmed, EMBASE, and the Cochrane Library from inception to 14 January 2019. Peer-reviewed studies of all types (*in vitro* studies, animal studies, and human trials) were selected.

Results and Discussion: The systematic literature search identified 16 clinical trials, 4 animal studies, and 64 *in vitro* studies. The effect of cruciferous vegetables or ITC intake on breast cancer survival was controversial and varied greatly across human trials. Most of these trials were observational studies conducted in specific regions, mainly in the US and China. Substantial evidence from *in vitro* and animal studies was obtained, which strongly supported the protective effect of ITCs against breast cancer. These studies showed sulforaphane and other ITCs reduced cancer cell viability and proliferation via multiple mechanisms and pathways. Isothiocyanates inhibited cell cycle, angiogenesis and epithelial mesenchymal transition, as well as induced apoptosis and phase II carcinogen detoxifying enzymes. Benzyl isothiocyanate showed a significant inhibitory effect on breast cancer stem cells, a new dimension of chemo-resistance in cancer treatment. Sulforaphane and other ITCs displayed anti-breast cancer effects at variable range of concentrations and benzyl isothiocyanate appeared to have a relatively smallest IC_{50}. In summary, current preclinical evidence strongly supports the role of ITCs as potential therapeutic agents for breast cancer, either as adjunct therapy or combined therapy with current anti-breast cancer drugs, with sulforaphane displayed the greatest potential.



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Pharmacokinetics of Treosulfan in paediatric patients

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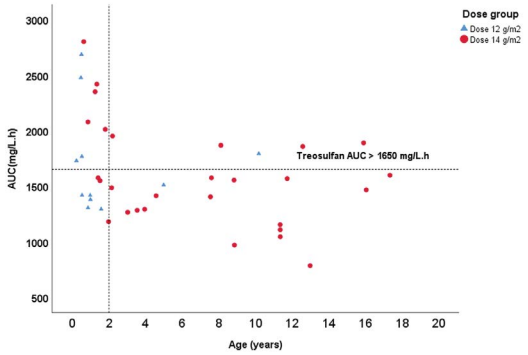
Introduction: Treosulfan is an alkylating agent that is used in blood or marrow transplantation conditioning regimens for the treatment of malignant and non-malignant diseases in children. The standard dose of 14 g/m^2 is often modified for children < 1 year to 12 g/m2 or lower; exposure above 1650 mg/L.h has been associated with increased mucosal and skin toxicity (Van der Stoep et al, 2017).

Aim: To examine the impact of age on exposure to treosulfan in paediatric patients.

Methods: Treosulfan (12 to 14 g/m2) was administered to 39 children age range (0.22 - 17 years) over 1.5 to 4 h. The sum of treosulfan and epoxy metabolites were measured in 5 to 8 acidified lithium heparin blood collected at timed intervals after the infusion using an HPLC-UV assay. Pharmacokinetic parameters (AUC, CL, V_{ss} and $t_{1/2}$) were determined using Kinetica (v4) software and compared between 17 children (< 2 years) and 22 older children (≥ 2 years) using the Mann-Whitney test.



Results: Treosulfan CL, V_{ss}, and $t_{1/2}$ were significantly lower in children < 2 years: CL was median (interquartile range (IQR)) 3.06 (2.13 – 3.84) versus 7.78 (6.97 – 11.49) L/h (n = 39; p < 0.001); Vss was 7.18 (5.06 – 8.47) versus 22.2 (15.9 – 3) L (n = 39; p < 0.001) and t1/2 was 1.38 (1.28 – 1.61) versus 1.68 (1.44 – 1.86) h (n = 39; p < 0.001) in the < 2 year group and ≥ 2 year groups, respectively. Children < 2 years had significantly higher treosulfan AUC compared with children > 2 years (median (IQR): 1730 (1398 -2385) versus 1477 (1237 -1647) mg/L.h, (n = 39; p < 0.05). 14 children had treosulfan AUC > 1650 µM.min; 9 of which were < 2 years.

Discussion: Treosulfan dosing requires further tuning in patients < 2 years to ensure exposure is equivalent across all paediatric age ranges, and to minimize the prevalence of mucosal and skin toxicity.

Van der Stoep E (2017) Br J Haematol 179:772 – 780.

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Deconvolution of apparent CYP2D6 induction by rifampicin using a extracellular vesicle derived biomarkers

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Introduction: Phenotyping analyses performed using metoprolol, debrisoquin and dextromethorphan pre- and post- rifampicin dosing have demonstrated changes in probe AUC and/or parent to metabolite ratios suggestive of CYP2D6 induction by rifampicin. These data are inconsistent with limited tissue biopsy data that demonstrate a lack of effect of rifampicin on the abundance of hepatic or intestinal CYP2D6 protein and mRNA.

Aims. Evaluate the impact of rifampicin on CYP2D6 expression and activity in humans using phenotyping and small extracellular vesicle (sEV) derived biomarker approaches.

Methods: CYP2D6 phenotype was evaluated on the basis of dextromethorphan (DXM) AUC and dextrorphan (DXO) to DXM ratio in a cohort of healthy males pre- (Day 1) and post- (Day 8) a seven day course of rifampicin. sEV derived CYP2D6 protein expression and *ex vivo* sEV catalysed conversion of DXM (25 µM) to DXO in the presence and absence of the selective CYP3A4 inhibitor CYP3Cide (0.05 µM) were evaluated using global sEV isolated from pre-dosing serum samples on Day 1 and Day 8.

Results: Consistent with prior phenotyping analyses, dosing of rifampicin for seven days resulted in a mean 1.34-fold reduction in DXM AUC from 1562 to 1166 µg/L/hr, and corresponding 1.35-fold increase in 3hr serum DXO:DXM ratio. Similarly, for incubations performed in the absence of CYP3Cide, there was a 1.36-fold increase in the mean *ex vivo* rate of DXM demethylation from 10.12 pmol/min/mL on Day 1 to 13.75 pmol/min/mL on Day 8. However, there was no difference in the *ex vivo* rate of DXM demethylation between Day 1 (8.92 pmol/min/mL) and Day 8 (8.68 pmol/min/mL) when incubations were performed in the presence of CYP3Cide. Analysis of sEV derived CYP2D6 protein abundance pre- and post- rifampicin dosing similarly demonstrated no change in CYP2D6 expression.

Discussion: The apparent induction of CYP2D6 expression by rifampicin reported on the basis of reduction in probe exposure in an artefact changes in the activity of minor metabolic pathways (CYP3A4 catalysed metabolism for DXM).

Berger el at (2018) Front Pharmacol 9: 774
Rodrigues and Rowland (2019) Clin Pharmacol Ther 105 1407

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Characteristics of extracellular vesicles secreted by HepaRG following removal of media component

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Introduction: HepaRG is a hepatocyte cell line used extensively as an in vitro model to assess the activity of drug-metabolising enzymes. It expresses a full array of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes at comparable levels to primary human hepatocytes and has intact response elements including PXR, CAR and PPARa. Small extracellular vesicles (sEV) are a heterogeneous family of membrane encapsulated particles (50 to 1000nm in diameter) that are released from cells both in vivo (i.e. into biofluids such as blood and urine) and during cell culture (i.e. into culture media). sEV contain mRNAs, miRNAs and proteins that reflect the cell of origin, and as such represent a potential novel source of minimally invasive biomarkers.

Aims. To characterise sEV released by HepaRG cells following the removal of media components.

Methods: HepaRG cells were differentiated and grown according to the manufacture's instructions. In order to assess sEV characteristics DMSO and FBS were removed from the media as per the protocol for CYP induction studies. During this phase media was collected and replenished after 24 and 48 hrs. sEV were isolated from the harvested media by differential ultracentrifugation at speeds of 1,500, 10,000 and 100,000 g respectively. The sEV pellet was resuspended in 40 µL of PBS and store at -80°C until use. sEV characteristics (abundance and size distribution) were evaluated by nanoparticle tracking analysis.

Results: The mean size and abundance of sEV isolated from media collected 24 hr after transition of HepaRG cells to induction media (97.55 nm and 3.9 x1010 particles/mL) was significantly different to that of the sEV collected prior to media transition (148.7 nm and 2.35 x1010 particles/mL) and those collected 48 hr after the media transition (135.31 nm and 8.08 x109 particles/mL) ($p < 0.05$). Similarly, the distribution of particle sizes for sEV collected 24 hrs after transition of HepaRG cells to induction media (87.8 to 104.6 nm) was more homogenous than that of the sEV collected prior to transition (134.6 to 174.5 nm) or those collected 48 hrs after transition (114 to 152 nm).

Discussion: This study demonstrated acute changes in the profile of sEV released from HepaRG cells in response to changes in media conditions.

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The effect of chronic polypharmacy, monotherapy and deprescribing on the kidneys of aged mice

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Introduction: Polypharmacy (use of ≥5 medications) with increasing DBI (Drug Burden Index or a measure of total exposure to anticholinergic and sedative medications) in older adults is associated with functional impairments, which may be reversed with deprescribing (withdrawal). The effect of polypharmacy and deprescribing on the kidneys, which are major organs involved in drug clearance, remains unclear.

Aims. We aim to investigate the effect of chronic polypharmacy, monotherapy and deprescribing on the kidney function and histology in aged mice.

Methods: At 12 months, healthy male C57BL/6 mice received control or treatment diet with therapeutic doses of medications (~±10-30%). The regimens consisted of Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram), or single medication from High DBI diet (n=40/group). At 21 months, mice were selected to continue treatment or undergo deprescribing (n=20/group). At 26 months, kidneys, serum and urine samples were collected for histology and biochemistry tests (n=4-15/group).

Results: Compared to control, all treatments had no difference in kidney/body weight ratio, kidney weights, serum cystatin C and creatinine levels, and urinary creatinine/albumin ratio, creatinine and albumin. Deprescribing had no effect on the above markers. Compared to control, Zero DBI, Low DBI, High DBI and metoprolol groups presented with elevated blood urea nitrogen (BUN) levels, which were reversed with deprescribing ($p<0.05$). Preliminary results (n=3/group) showed no change in tubular atrophy score in any treatment groups compared to control.

Discussion: Our results show that chronic polypharmacy and monotherapy of selected medications do not contribute to renal impairments in old age. Zero DBI, Low DBI, High DBI and metoprolol may increase BUN levels, but can be reversed with deprescribing. Further histological analysis will confirm these outcomes.

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Sulfasalazine: a controversial pro-drug to treat rheumatic diseases

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Introduction: Sulfasalazine (SSZ) was initially introduced as a pro-drug to treat bowel infections believed to cause rheumatoid arthritis (RA), ankylosing spondylitis (AS) and ulcerative colitis (1). Nowadays, SSZ is also used to treat psoriatic arthritis. It is bio-activated by bacterial azoreductase(s) within the anoxic lower gut, yielding 5-aminosalicylate (5-AS), an antoxidant and sulfapyridine (SP), an antibacterial. There was a long-continuing controversy about which of these two metabolites was the active anti-arthritic agent.

Methods: SSZ and its two major enteric metabolites: SP (aka M&B693), and 5-AS were tested for growth inhibitory activity - using disc diffusion and liquid dilution MIC techniques - against bacterial triggers of (a) rheumatoid arthritis (RA) (Proteus spp)., (b) ankylosing spondylitis (AS) (*Klebsiella pneumonia*), (c) multiple sclerosis (MS) (*Acinetobacter baylyi(2)* and *Pseudomonas aeriginosa*) and (d) rheumatic fever (*Streptococcus pyogenes*). SP and 5-AS were also tested in combination. ΣFIC analysis was used to determine the class of interaction and isobologram analysis identified the ideal ratios to achieve significant potentiation.

Results: SSZ was an effective (pro)-drug, with good growth inhibitory activity against multi-drug resistant strains of *P. mirabilis*, *P. vulgaris*, *K. pneumonia*, *A. baylyi*, *P. aeruginosa* and *S. pyogenes*. SP was more effective than SSZ (or 5-AS), with up to 8 times greater potency against some bacteria. Notably, the inhibitory activity of SP was potentiated when tested in combination with 5-AS. Indeed, synergy (defined as ≥4 fold increase in efficacy) was noted against *P. vulgaris*, whilst additive effects (2-4 fold increases in efficacy) were noted against *P. mirabilis*, *K. pneumonia* and *S. pyogenes*. Isobologram analysis of SP/5-AS combination against *P. vulgaris* determined that all ratios containing ≤60% SP were synergistic.

Conclusion. Generation of SP and 5-AS from the SSZ pro-drug within the lower GI tract may create a combinational therapy with greater efficacy than the pro-drug SSZ, against the bacterial triggers of several chronic inflammatory diseases.

(1)Swartz N (1942). J Int Med 110:577-598. (2) Whitehouse MW (2015) Inflammopharmacol 23:371-374.

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Development of a cell-based assay for screening of P2X1-purinoceptor antagonists for use in male contraception

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Introduction: Male contraceptives are limited to condoms or vasectomies, both of which come with drawbacks that reduce compliance. Therefore, new male contraceptives are needed to assist in reducing high unintended pregnancy rates. Dual knockout of P2X1-purinoceptors and α_{1A}-adrenoceptors exhibited complete infertility in male mice while normal behaviour and physiology was maintained, and sperm viability retained (White et al, 2013). 1A-adrenoceptor antagonists are well researched and available on the market, however current P2X1-purinoceptor antagonists are not suitable for human use. Hence, the next step for a male contraceptive pill is to develop a suitable small molecule P2X1-purinoceptor antagonist.

Aims: To optimize a cell-based system for expression of P2X1-purinoceptors and development of a reliable cell-based assay to assess activity of newly synthesized P2X1-purinoceptor antagonists.

Methods: Gateway technology was used to prepare P2X1 containing constructs. Transfections were performed using a cationic lipid method and the Flp-In system. The developed P2X1 expressing HEK293 Flp-In T-REx (Tet-On) cell line was used to measure intracellular Ca^{2+} influx on a FlexStation 3 multi-mode microplate reader and FDSS/µCELL Functional Drug Screening System. A set of 11 novel compounds were analysed in the cell-based assay to determine and compare P2X1 antagonistic activity, using suramin as a standard.

Results: A HEK293 Flp-In T-REx cell line was developed using the pcDNA5/FRT/TO-DEST P2X1 containing construct. ATP produced a non-specific response which was similar in transfected and naïve cells. In contrast, α,β-methylene ATP produced responses that yielded a pEC_{50} of 7.05M (7.33-6.79, 95% CI, n=8) in cells expressing P2X1-purinoceptors, while responses in naïve cells were negligible. Novel compounds displayed moderate non-competitive antagonist activity against α,β-methylene ATP in this cell-based assay.

Discussion: In comparison with the developed rat vas deferens electrical stimulation assay the novel compounds were consistent with a few exceptions. The development of a cell-based bioassay for P2X1-purinoceptor activity is a useful asset for quickly analysing potency at the human P2X1-purinoceptor without confounding factors.

1. White C et al. (2013) Proc Natl Acad Sci 110:20825-20830.

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Developing Cannabinoids to target T-type calcium channels involved in epilepsy.

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Introduction: T-type calcium channels play critical roles in brain function and altered behavior due to injury or genetic mutations can cause many diseases including epilepsy[1,2]. Cannabis-based medications are now approved in Australia for treatment of epilepsy and although knowledge of the human *endo*cannabinoid system has increased, large knowledge gaps remain surrounding the exact mechanisms by which cannabinoids exert their therapeutic effect3.

Aims: Identify natural and synthetic cannabinoids that regulate T-type calcium ion channels associated with epilepsy and determine how cannabinoids modulate these channels and define the regions responsible for their action.

Methods: A combination of fluorometric (FLIPR) assays and patch clamp electrophysiology were used to screen natural and synthetic cannabinoids for their ability to block or modulate T-type calcium channels. Experiments used HEK293 Flp-In T-REx cells stably expressing $hCa_v3.1$, 3.2 and 3.3.

Results: CBGA (cannabigerol acid) was the most potent natural cannabinoid to block T-type hCaV3.1 and 3.2 with >90% block of peak channel current at 10 µM. Synthetic Cannabinoids' MDMB-CHMICA and AMB CHMINACA also blocked peak channel current >90% at 10 µM for both T-types, however these drugs only partially blocked $hCa_v3.3$ (30%). Overall, most natural and synthetic cannabinoids tested had far less potency on $hCa_v3.3$ suggesting that cannabinoid binding to 3.1 and 3.2 involves a unique site or drug/protein interaction not found in $hCa_v3.3$.

Discussion: $hCa_v3.3$ shares almost 90% sequence homology with the other 2 T-types with largest differences found in the proposed gating mechanism that give $hCa_v3.3$ its slower gating kinetics. Selective cannabinoid binding and altered kinetics of $hCa_v3.1$ and 3.2 versus $hCa_v3.3$, suggests binding of Cannabinoids to T-types involves the gating region or the different conformational states brought on by altered gating kinetics. Further research will help elucidate the binding mechanisms and sites and potentially lead to development of better Cannabinoid therapies in the future.

1. Catterall WA et al (2005). *Pharmacol Rev* 57: 411-425.
2. Bladen, C et al., (2014). *ACS Chemical Neuroscience* 6: 277-287.
3. Devinsky O et al., (2014). *Epilepsia* 55: 791-802.

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

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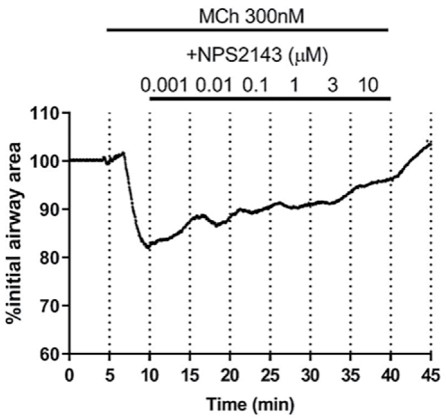
Introduction: The CaSR detects changes in extracellular calcium to maintain homeostasis. This putative drug target is upregulated in asthma, and allosteric agonists for CaSR, such as the polyamines, induce bronchoconstriction. Further, negative allosteric modulators (NAMs; e.g. NPS2143) reduce airway 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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Using untargeted metabolomics to discover the killing mechanism of Ceftazidime-avibactam against multidrug resistant *K. pneumonia*

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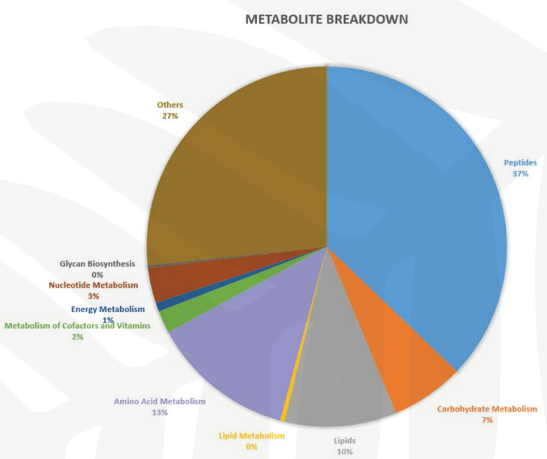
Introduction: Ceftazidime (CAZ)-avibactam (AVI), a new combination, has proven efficacious against such multidrug resistant Gram-negative pathogens including *A. baumannii, K. Pneumoniae* and *P. aeruginosa* strains. The compounds individually have well-characterised mechanisms of action (MOA), thus proposing highly probable synergy for the combination. Individually, each compound has minimum inhibitory concentrations (MICs) of 128mg/L (CAZ) and >128mg/L (AVI). When used together in a ratio of 4 (CAZ): 1 (AVI), the MIC is reduced to 8mg/L.



Methods: Static timekill assays using sub-MIC drug concentrations (2.5mg/L) were conducted and optimised against *K. pneumoniae* strain KPMA100-09. Samples for metabolomic analysis were taken 1h, 3h, 6h and 24h after adding the compounds, purified, and metabolites extracted. Metabolites were quantified using LC-MS and raw data from the LC-MS was converted to metabolites using IDEOM. The significance of each metabolite in the KPMA100-09 pathways was evaluated against metabolomics databases.

Results: Analysis at each time point showed time-dependent killing of the bacteria, with maximal killing at 3 hours, where we observed changes in the peptidoglycan biosynthesis, amino-sugar biosynthesis as well as pentose phosphate pathways. The pentose phosphate pathway is of particular significance as downstream metabolites such as erythrose-4-phosphate are involved in the synthesis of lipopolysaccharide, a key component of peptidoglycan. These perturbations prevent important processes in bacterial cell division, thus killing the bacteria.

Discussion: Understanding the pathways being affected by such a therapeutic intervention will also be helpful in predicting the emergence of further resistance down the line.

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Dissecting mechanisms of fenestration dynamics in liver sinusoidal endothelial cells.

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Introduction: Liver sinusoidal endothelial cells (LSECs) are a filtration interface between plasma and the space of Disse, via nanopores - “fenestrations”. These dynamic structures, which open and close, can be influenced by various agents. In addition, their number and diameter are reduced with ageing. Since fenestrations are conduits for drugs from the plasma to the hepatocytes, this age-related fenestration loss has serious consequences for drug metabolism. The mechanics and structure of fenestrations, however, remain to be elucidated.

Aims: The aim of this study is to find the mechanism(s)/protein(s) and substance(s) that influence and/or control the dynamics of fenestrations.

Methods: The influence of agents such as sildenafil[a] and sildenafil analogues on LSECs morphology and scavenging were analysed with super resolution microscopy, and endocytosis assays with ^{125}I-FSA. Additionally, fluorescent antibody staining for surface proteins involved in redox reactions were performed to determine their location relative to fenestrations, since hydrogen donors appear to enhance LSEC fenestrations.

Results and Discussion: Preliminary results show increases in porosity are elicited by sildenafil and analogues without any alterations in endocytosis. The redox enzymes NQO1 & GLRX3 show discrete patch-like distribution, some of which co-localise with fenestrated sieve plates, while CYB5R3 & GST-Pi have a more general distribution. Further studies using immuno-TEM will determine the close association these enzymes with fenestrae, and if they have a potential role in regulating LSEC porosity.

[a]Hunt et al. (2019) Manipulating fenestrations in young and old liver sinusoidal endothelial cells, *Am J Physiol Gastrointest Liver Physiol 316: G144 doi:*10.1152/ajpgi.00179.2018.

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Live cell imaging: An assessment tool for breast cancer cell death

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Introduction: Breast cancer cells develop a variety of mechanisms to bestow resistance to cell death inducers, including those which induce apoptosis, necrosis and oncosis. Modulation of calcium signalling could potentially circumvent these pathways to induce breast cancer cell death. One challenge in defining the potential of targeting the calcium signal to promote breast cancer cell death relate to limitations in assessing the calcium signal during key stages in breast cancer apoptosis, necrosis and oncosis.

Aims. To evaluate the use of live cell imaging in a 96 well plate format to identify key stages of breast cancer cell death in MCF-7 and MDA-MB-468 breast cancer cells.

Methods: MCF-7 and MDA-MB-468 breast cancer cells were plated onto 96 well plates and assessed over 24-48 h, during treatment with the cell death inducers ethanol or staurosporine. Cells were imaged every 10-15 min using bright-field imaging via JuLI™ Stage (NanoEntek).

Results MCF-7 and MDA-MB-468 cells exhibited distinct temporal and morphological changes with ethanol and staurosporine. MDA-MB-468 breast cancer cell morphology was more sensitive to ethanol than MCF-7 cells, with almost immediate membrane ruffling, then subsequent cell rounding or apoptotic body formation. Rapid cellular swelling and oncosis were also observed when exposed to higher ethanol concentrations. In MDA-MB-468 cells, treatment with staurosporine also produced similar morphological changes as ethanol (eg. cell rounding). In contrast, staurosporine induced pronounced cellular protrusions in MCF-7 cells. The time to maximal cellular protrusion was also concentration dependent.

Discussion: The pronounced differences in the nature of morphological and temporal changes induced by different cell death stimuli and between different breast cancer cell lines can be assessed using high throughput imaging. This can inform selection of temporal windows for the assessment of calcium signal changes during key stages of breast cancer cell death.

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Nanomedicine based delivery of metformin and nicotinamide mononucleotide (NMN) to single cell types of the liver via oral administration

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Introduction: This study firstly demonstrates the targeted delivery of silver sulfide quantum dots (QDs) following oral administration to the liver sinusoidal endothelial cells (LSECs) and hepatocytes *in vitro* and *in vivo*, using radiolabeled tracking, fluorescent marking and transmission electron microscopy. We then aimed to show improved bioavailability and biological effects of two agents, metformin and NMN, due to nanomedicine/QD transport.

Methods: Radiolabeled metformin or NMN was bound to QDs followed by biopolymer coatings. Coatings were used to promote specific cell type receptor mediated uptake as well as regulation of the endocytosis pathway. Treatment of cell lines and oral gavage of mice with nanomedicines was performed.

Results: Metformin and NMN have poor (8-12%) liver/hepatocytes bioavailability in mice; conjugation with QDs improves delivery to hepatocytes 5-fold (45-65%). LSECs with biopolymer coatings demonstrate a 50-fold greater delivery of drug agents both *in vitro* and *in vivo* due to changing endocytosis pathways and avoiding reliance on cation uptake channels. Drug treatment of cell lines (SK-Hep1 and HepG2) demonstrate 100-fold lower dosages are required to promote similar activation in pAMPK/AMPK, peNOS/eNOS, SIRT1 and pIRS-1/IRS-1. *In vivo* oral treatment of mice demonstrates similar activation pathways in the liver over 24 hrs. These mice do not demonstrate changes in liver, small bowel, spleen or kidney histology, nor changes in AST/ALT. Finally, radiotracking of QDs shows 85% are excreted within 24 hrs. In conclusion, we have shown high specificity targeting of the liver and single cell types hepatocytes and LSECs by QD based nanomedicines for the delivery of metformin and NMN *in vivo* via oral pathways.

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Unravelling signalling mechanism of peptide mimetic at the relaxin receptor RXFP1 in human cardiovascular cells

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Introduction: Relaxin mediates vasodilatory, anti-fibrotic, anti-inflammatory and post-injury healing effects via its cognate receptor, RXFP1. Recombinant H2 relaxin has demonstrated considerable promise as a treatment for acute heart failure (AHF). While it did not meet primary endpoints in a Phase IIIb study, patients showed improvements in markers of cardiac, renal and hepatic damage consistent with the prevention of organ damage. We recently developed a H2 relaxin mimetic peptide, B7-33, and showed it has cell-specific actions (Hossain et al., 2016. Chem. Sci. 7:3805-3819).

Aims. To use the relaxin mimetic peptide B7-33 to better understand signalling at the RXFP1 receptor with particular emphasis on the cardioprotective effects of relaxin.

Methods: We compared B7-33 and H2 relaxin-mediated signalling in HEK cells overexpressing RXFP1 (HEK-RXFP1) with cell lines endogenously expressing RXFP1. We used primary human cells relevant to the clinical actions of H2 relaxin including human cardiac fibroblasts and human endothelial vascular cells. We applied Surefire, Alphascreen or HTRF assays to investigate cAMP, cGMP and MAPK signalling.

Results: B7-33 was a weak agonist in HEK-RXFP1 cells and some native cells but exhibited equipotent activity to H2 relaxin in other native cells including human cardiac myofibroblasts (HCF). B7-33 stimulated p-ERK1/2 and cGMP in HCF with equipotent activity to H2 relaxin. Interestingly, in primary human umbilical vein endothelial cells (HUVECs) B7-33 only stimulated cGMP pathway and showed no activity at cAMP whereas H2 relaxin is a potent activator of both cAMP and cGMP accumulation in these cells.

Discussion: We have obtained a first evidence of ligand-directed signalling bias of peptide mimetic B7-33 as compared to H2 relaxin. Further investigation of B7-33 versus relaxin activities in human vascular cells will provide valuable information of their signalling mechanism relevant to cardiovascular protective actions of relaxin.

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Oxidative stress and calcium signalling in breast cancer and human fibroblast cell co-cultures

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Introduction: The tumour microenvironment (TME) is a key regulator of breast cancer progression. Recent studies suggest that fibroblasts in the TME may undergo calcium signalling remodelling and that specific calcium channels may promote tumorigenic pathways involved in oxidative stress resistance. Genetically encoded calcium indicators and automated epifluorescence microscopy may help define the complex interplay between cancer cells and the tumour stroma during oxidative stress.

Aims. To compare Ca^{2+} homeostasis and cell survival in breast cancer cells during oxidative stress in coculture with human mammary fibroblasts.

Methods: MDA-MB-231 breast cancer cells stably expressing the green genetically encoded Ca^{2+}indicator GCaMP6m were cultured with or without HMF3S breast fibroblasts stably expressing the red genetically encoded Ca^{2+} indicator jRCaMP1b. An ImageXpress system was used to assess cell survival and cytosolic free Ca^{2+} ($[Ca^{2+}]CYT$) levels in the presence of H2O2 (0 – 10 mM) over 14 hours.

Results: H_2O_2 produced a concentration dependent reduction in MDA-MB-231 cell viability. Breast cancer cells were more resistant to H2O2 induced cell death and $[Ca^{2+}]CYT$ increases when co-cultured with HMF3S cells.

Discussion: Fibroblasts of the TME may protect breast cancer cells against the effects of oxidative stress through modulation of Ca^{2+} homeostasis.

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Interleukin-18: a novel target for drug discovery to treat hypertension?

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Introduction: Hypertension is a major risk factor for cardiovascular and renal diseases. Currently, therapies target the symptoms (i.e. elevated blood pressure) rather than addressing the mechanisms underlying the condition. Recently, the dysregulation of the immune system has emerged as a potential pro-hypertensive mechanism.

Aims. To (1) identify, prioritise and validate a potential novel drug target for the treatment of hypertension and (2) evaluate therapies against this target for potential drug re-purposing in hypertension.

Methods: Drug Research Advisor – Target Druggability (Clarivate Analytics) was used to identify novel targets as candidates for re-purposing drugs to treat hypertension based on the weight of genetic evidence, biomarker use and associated drug pipeline data. Cortellis Drug Discovery Intelligence (Clarivate Analytics) and OFF-X (Bioinfogate) were used to further assess the suitability of a selected candidate target based on development status, experimental pharmacology, experimental models used and safety/toxicology data of drugs against the identified drug target.

Results: Interleukin (IL)-18, a type of pro-inflammatory cytokine that influences T and natural killer cell responses, was identified as one of the top 3 immune-related candidate targets for hypertension. In further support of IL-18 as a druggable target, this cytokine has been used as biomarker for various roles including diagnosis of hypertension (56%) and as a risk factor for this disease (22%) in both experimental models and humans. Analysis of the drug landscape for IL-18 demonstrated 13 therapies (9 in biological testing/preclinical and 4 in clinical development phase) used to treat various inflammatory conditions, all highlighting anti-IL-18 as their mechanism of action. For these therapies, cell lines were used to measure IL-18 affinity in vitro and efficacy of IL-18 inhibition was further validated in 3 experimental disease models. Treatment with an IL-18 binding protein, Tadekinig Alfa, in a Phase 2 clinical trial involving patients with adult-onset Still's disease was associated with improvement of symptoms of the condition (reduction in swollen joint count) and minimal adverse events of which were considered mild or moderate.

Discussion: IL-18 represents a novel drug target to treat hypertension. Therapies targeting IL-18 currently in the drug pipeline for various inflammatory conditions appear efficacious in both pre-clinical and early phase clinical settings, are well tolerated in patients and thus have the potential to be re-purposed to treat hypertension.

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Assessment of Ca^{2+} channels and pumps in brain-seeking breast cancer cell lines.

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Introduction: Metastasis of breast cancer cells to the brain is associated with a rapid reduction of quality of life and in all cases is associated with reduced life expectancy. Resistance to therapy in breast cancer brain metastasis is associated with specific alterations in cell signalling pathways. Preliminary studies using clinical samples suggest that some Ca^{2+} channels may have altered expression and/or activity in breast cancer cells that have metastasised to the brain. Breast cancer cell lines that have been selected for their ability to grow in the brain such as MDA-MB-231-Br and BT-474-Br have been used in a variety of studies to help identify genes that may be important in brain metastasis. However, there have been very few studies assessing Ca^{2+} channels and pumps using these experimental models.

Aims. To assess mRNA levels of Ca^{2+} pumps and channels in breast cancer brain metastases-derived cell lines and their parental counterparts.

Methods: RNA was isolated from three independent passages of the brain seeking breast cancer cell lines BT-474-Br1 and MDA-MB-231-Br3 and their parental counterparts BT-474 and MDA-MB-231. Real time PCR was used to compare levels of a suite of Ca^{2+} channels and pumps.

Results: MDA-MB-231-Br3 have significantly different levels of the mechanosensitive ion channel PIEZO1 and the Ca^{2+} store release channels IP_3R1 and IP_3R3 compared to parental MDA-MB-231 cells. However, there were no significant changes in BT-474-Br1 compared to its parental line.

Discussion: Some breast cancer cells with an enhanced ability to metastasise to the brain may have altered levels of specific Ca^{2+} channels. Such changes may be related to the cell line model, including the molecular subtype and the number of selection rounds used to enhance brain seeking abilities.

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Expression of Wnt signalling components and cytotoxicity of Wnt inhibitors in colorectal cancer HCT-15 and HCT116 cells

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Introduction: Targeting abnormal Wnt signalling pathways in colorectal cancer is a credible strategy of developing new anticancer drugs. Wnt inhibitors ICG001 (β-catenin/TCF inhibitor), XAV939 (tankyrase inhibitor) and PNU74654 (β-catenin/TCF inhibitor) are under preclinical test in different cancer types, but not systemically in colorectal cancer that frequently harbours APC and β-catenin mutations.

Aims. To determine the expression of major Wnt pathway components in colorectal cancer cells; and the potency of Wnt inhibitors as single agent and in combination with chemotherapeutic drugs.

Methods: mRNA of Wnt components was measured by RT-PCR; Protein levels were measured by Western blotting. The single agent cytotoxicity and synergisms were examined in human colorectal cancer HCT-15 and HCT116 cells by measuring the combination index (CI) using Chou-Talalay method and Compusyn program.

Results: Expression of major Wnt pathway components differs in colorectal cancer cell lines. At mRNA level, FZD7, LPR6, DVL1 & CTNNB1 were detected in HCT-15 cells; FZD7 & CTNNB1 were detected in HCT116 cells. At protein level, LRP6, FZD7, DVL2 & active β-catenin were detected in HCT-15 & HCT116 cells. ICG001, XAV939 and PNU746454 were less cytotoxic than oxaliplatin (OXL), fluorouracil (5-FU) or irinotecan (IRI), with IC50 values measured as 17±5, 62±10 & 97±2.3 μM in HCT-15 cancer cells; 27±11, 75±7 & 98±26 μM in HCT116 cells, respectively. In combination study, XAV939 displays synergism with irinotecan in both cell lines, PNU74654 with 5-FU in HCT-15 cells, and ICG001 with oxaliplatin in HCT116.

Discussion: Key drug targets of Wnt pathways are expressed in colorectal cells, including FZD7 and active β-catenin, but not TCF, which needs to be considered when designing inhibitors. Cytotoxic synergism is observed between specific Wnt inhibitors and chemotherapeutic drugs, which warrants further *in vivo* tests. Supported by Royal Hobart Hospital Research Foundation and Cancer Council Tasmania.

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Developing a systematic efficient method to determine synergistic 3 herb combinations in the search for osteoarthritis treatments

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Introduction: Osteoarthritis (OA) is a common degenerative chronic articular disease. Inflammation in synovium and cartilage play a crucial role in the pathophysiology of osteoarthritis. The use of multi-herb combinations is common in herbal medicine practice, and widely gaining attention to treat OA, aiming to enhance therapeutic efficacy by synergistic action.
Aims. The current study aimed to develop and evaluate a novel simple activity balancing method to screen for synergism in a three-herb combination of herb A, B and C to treat OA through using tumor necrosis factor (TNF-α) inhibition using in vitro cell models of IL-1β stimulated chondrocytes and LPS and INF-γ-stimulated RAW246.7 cell lines.

Methods: The study was carried out in two stages. Firstly, the concentration-effect curves for the three herbs were established. Secondly, the drug combination study was performed where the doses of the individual herbs were normalised to the maximal safe concentrations, resulting in each herb contributing equally to activity at a 1:1:1 ratio. The ratios were designed to cover all combinations from 9:3:1 giving 19 ratios. Combination index (CI) values were calculated using Calcusyn software and used to determine synergistic, agonistic or antagonistic interactions among the studied combinations.

Results: Combinations contained 3:1:1 and 3:1:1, 9:1:1 based on maximum safe concentrations respectively in chondrocytes and RAW cells for herb A: B: C was found to be optimal ratios and showed greater potencies than the individual herbs.

Discussion: This study demonstrated that the three herbs in the formula exert synergistic effects, which is beneficial for the treatment of OA. There were no predictable trends observed with the combinations, meaning that mathematic prediction models may be inadequate to guide complex 3 herb combinations. Furthermore, the study design may serve as a suitable method for evaluating synergism in multi-herb combinations.

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Development of a safe and effective painkiller from the spider venom peptide Pn3a

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Introduction: Pain is the leading cause of disability in the developed world but remains a poorly treated condition. Existing analgesics often suffer from poor tolerability, abuse potential and a lack of broad efficacy. One of the most promising targets for effective and safe pain treatment is Nav1.7, a neuronal voltage-gated sodium channel (Nav) subtype. Genetic studies support a key role of Nav1.7 in pain since gain-of-function and loss-of-function mutations lead to pain syndromes and insensitivity to pain, respectively. We identified the tarantula venom peptide μ-theraphotoxin-Pn3a (Pn3a) as potent and selective Nav1.7 blocker with effective analgesic properties.

Aims. The aim of this project was to identify the pharmacophore of Pn3a and to develop a more efficacious and safe Pn3a-analogue as lead analgesic compound.

Methods: Solid-phase peptide synthesis was used to produce Pn3a-analogues and folding was confirmed via NMR. Fluorescence imaging and patch-clamp experiments were performed to pharmacologically characterize potencies at hNav1.1-hNav1.8. Surface plasmon resonance was used to examine peptide-lipid bilayer interactions. Improved analogues were tested in mouse models of OD1-induced Nav1.7-mediated pain and in a post-surgical pain model.

Results: Mutations of arginine 23, lysine 24 and several hydrophobic residues in Pn3a decreased inhibition of Nav1.7 *in vitro*. Mutations at four different acidic residues increased potency with two mutants also showing improved selectivity and membrane affinity. Especially Pn3a[D8N] was more potently analgesic and more efficacious than Pn3a in the *in vivo* OD1 and the post-surgical pain models without causing side effects after systemic administration.

Discussion: Pn3a is a unique pharmacological tool to define the role of NaV1.7 in pain pathways and has potential to become a blueprint for the development of novel pain treatments. The obtained information about the pharmacophore of Pn3a can be used to design analogues with desired pharmacological properties. Indeed, promising analogues with improved potency, selectivity and membrane binding were identified with one analogue showing improved analgesic efficacy *in vivo*, making it a promising lead candidate for further development of improved safe and effective painkillers.

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AG-OX, a potent inhibitor of interactions between S100A9 and RAGE/TLR4, selectively inhibits S100A9-positive triple-negative breast cancer.

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Introduction: S100A9 is protein consisting of 114 amino acid and has been reported to be found only in vertebrates. This is in myeloid cells, cancer cell and tumor stroma. It is abundant cytoplasmic protein in normal myeloid cells such as polymorphonuclear cells and monocytes. Apparently S100A9 proteins can perform a wide plethora of intra- and extracellular functions via activation of the receptor advanced glycation end products (RAGE) and toll-like receptor 4 (TLR4) dependant signalling cascades and potentially other signalling pathways. And S100A9 may affect tumor metastasis by influencing the process of angiogenesis.

Aims. The purpose of this study is to develop drugs that have selective anti-cancer efficacy in in-vitro and in-vivo by inhibiting interaction between S100A9 and RAGE in the triple-negative breast cancer cell (TNBC) expressing S100A9.

Methods: In the process of ProteinChip–based S100A9 inhibitor scanning, a solution of 100 μg/mL RAGE/TRL-MD-2 was spotted onto the ProteinChip and this chip was incubated overnight in a humidity chamber at 4°C. After extensive rinsing, the RAGE/TRL4 array was subsequently spotted with a mixture of Cy5-labeled S100A9 protein and oxyclozanide in a humidity chamber. The fluorescence intensity of the mixture was measured at a specific control spot and competitive inhibition was evaluated. In western blotting, total proteins were extracted using RIPA lysis buffer and sample were resolved by SDS-PAGE. It was performed using either anti-pERK, anti-PARP, or anti-TSP-1 antibodies.

Results: In ProteinChip assay, oxyclozanide strongly inhibited the interaction between S100A9 and RAGE and TLR-4 and selectively inhibited the proliferation of cancer cells in MDA-MB-468 of the S100A9-produced TNBC cell. In western blotting analysis, oxyclozanide decreased PERK expression in S100A9-exposed TNBC cells and increased the expression of the cleaved poly ADP-ribose polymerase (cleaved PARP) in relation to apoptosis induction. In order to assess angiogenesis in basic fibroblast growth factor (bFGF) – induced human umbilical vein endothelial cells (HUVECs), oxyclozanide inhibited the formation, multiplication, and movement of intravenous cells.

Discussion: Oxyclozanide was shown to have anti-cancer activity in cells and in preclinical TNBC models in which S100A9 was expressed. These data support the applicability of oxyclozanide for treatment of patients with TNBC.

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Biased agonism at chemokine receptors: critical role for the chemokine N-terminal region

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Introduction: Leukocyte trafficking in inflammation is stimulated by chemokines, expressed at the site of tissue injury, interacting with chemokine receptors, G protein-coupled receptors expressed in the leukocyte plasma membrane. Although chemokine receptors are popular targets for new anti-inflammatory drugs, successful development of such drugs is hampered by the complexity of chemokine-receptor networks. In particular, different chemokine ligands may exhibit biased agonism, i.e., activate the same receptor to induce distinct signalling pathways and downstream cellular effects.

Aims. We aimed: (1) to evaluate biased agonism by chemokine ligands at their shared receptor CCR1, implicated in various inflammatory diseases; and (2) to identify the structural features of chemokines affecting their differential activation of CCR1.

Methods: We compared the abilities of the chemokines CCL7, CCL8 and CCL15 to activate CCR1 using four different cell-based signaling assays: recruitment of βArrestin; G protein activation; inhibition of cAMP production; and phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). Biased agonism was evaluated using the Black and Leff operational model of agonism. Subsequently, the effects of N-terminal length and sequence were evaluated using chemokine variants and mutants.

Results: Compared to CCL15, CCL7 and CCL8 exhibited biased agonism towards cAMP inhibition and away from βArrestin 2 recruitment. N-terminal truncation of CCL15 or substitution of the CCL15 N-terminus with that of CCL7 yielded modified chemokines with similar biased agonism to CCL7.

Discussion: These results suggest that the interactions of the chemokine N-terminal region with the receptor transmembrane region play a key role in selection of receptor conformations coupled to specific signaling pathways.

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Development of a luciferase complementation method to investigate extracellular CXCR4 conformational changes.

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Introduction: The Nanoluciferase NLuc has been used to investigate many aspects of cell signalling including protein-protein interactions and ligand binding. Recently NLuc has been engineered as two self-complementing fragments, HiBiT and LgBiT, which can be used to investigate receptor internalisation. However we found that when using this method to investigate CXCR4 internalisation that antagonists of CXCR4 increased luminescence output suggestive of an increase in cell surface expression and therefore ligand induced inhibition of constitutive CXCR4 trafficking.

Aim: To investigate the mechanism by which antagonists of CXCR4 increase luminescence output in HiBiT/CXCR4 complementation assays.

Methods: HEK293 cells expressing exogenous, or CRISPR/Cas9 genome edited CXCR4 tagged on the N-terminus with HiBiT were generated. Assays were performed in live cells or membrane preparations. Luminescence was generated in the presence or absence of ligand by addition of purified cell impermeant LgBiT and furimazine. Light emissions were measured using a PHERAstar plate reader (BMG).

Results: In live cells expressing exogenous or genome-edited HiBiT/CXCR4, as well as membrane preparations expressing exogenous HiBiT/CXCR4 or in cells where endogenous CXCL12 had been knocked-out, AMD3100 and IT1t resulted in a concentration dependent increase in luminescence (n=4-5) following luciferase complementation. Together these data did not support a hypothesis of antagonist mediated inhibition of CXCR4 trafficking being the only driver of the increase in luminescence. However, we also found that the affinity of HiBiT-LgBiT complementation was reduced when HiBiT was fused to CXCR4 (Kd = 229.8 ± 37.2 nM versus 6.99 ± 0.45 nM for purified luciferase fragments only, n=5) and that in the presence of AMD3100 (1 μM) the affinity of complementation increased (Kd= 58.5 ± 9.6 nM)

Discussion: Here we demonstrate that fusion of HiBiT to CXCR4 reduces the affinity of HiBiT-LgBiT complementation and that ligand binding to CXCR4 likely drives a conformational change in the N-terminus that is more favourable for luciferase complementation. Exploiting these differences in HiBiT-LgBiT affinity provide an opportunity to investigate ligand-binding and extracellular conformational changes of HiBiT-tagged receptors in a simple luminescence assay.

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A traditional anti-inflammatory from Tasmania and Southern NZ: Mutton Bird Oil (MBO)

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Background: The mutton bird aka moonbird or short-tailed shearwater *Puffinus Tenuirostris* is a seabird belonging to the petrel family. It inhabits the shores and islands around Tasmania and also the southern coast of New Zealand. Traditionally the waxy red stomach oil (Yolla) in the young chicks has been harvested as a) a medication for dermal application b) dermal insulator (before wet suits were available) and c) water proofing agent for leather and wood. This study examined the anti-inflammatory, immunosuppressant and gastroprotectant activities of MBO in rats with experimental polyarthritis.

Methods: Chronic arthritis was initiated in rats by caudal injection of either delipidated dry *Mybact. tuberculosis* (*hominus*) dispersed in squalane or bovine type-II collagen dispersed in Freund's Incomplete Adjuvant (FIA). MBO obtained from a) Aboriginals on Flinders Island, Tas. and b) Maoris on Stewart Island NZ, was administered (i) orally to male Wistar rats for 14 days or (ii) transdermally (admixed with 20% isopropanol v/v) for four days to shaved dorsal skin (6 cm^2), after first expression of arthritis (usually Day 10).

Results: MBO oils (Tas & NZ) were effective anti-inflammatories suppressing arthritis development. Unlike many oils, they did not induce arthritis when used instead of squalane or FIA to initiate arthritis. MBO also protected rats from gastric injury caused by oral co-administration of aspirin (150 mg/kg) or ibuprofen (50 mg/kg). These activities of MBO were retained up to 20 years after harvesting.

Conclusion: The red astaxanthin content (ca.0.2% w/v) seems to be an effective antoxidant and preservative MBO is not gastrotoxic but a safe nutritional product deserving further clinical evaluation as a medicinal.

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Oxytocin-vasopressin ligand-receptor bias for Gq signalling: Man and mouse

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Introduction: The notion that oxytocin (OT) and vasopressin (AVP) each act at both OT and AVP receptors has become a prominent consideration in interpreting data from animal models on the behavioural effects of these neuropeptides in neuropsychiatric disorders (Song and Albers, 2018). However, there are currently only superficial models comparing the underlying receptor pharmacology across species.

Aims. Generate system-independent measures of pharmacological activity at human and mouse OTR and V1aR to better understand the pharmacodynamic parameters of this cross-talk.

Methods: Reproducible high and low levels of human or mouse OT or AVP1a receptors (h/m OTR/V1aR) were achieved via tetracycline-dependent control of expression in stably transfected HEK293 cells. Inositol monophosphate (IP1) accumulation assays were performed and data was fit to the Black and Leff operational model, yielding parameters of functional affinity (K_A) and efficacy (τ).

Results: For OT, the K_A were 550 nM, 74 nM, 469 nM and τ values were 24, 21, 2 at hOTR, mOTR, hV1aR, respectively. For AVP, the K_A values were 2431 nM, 168 nM, 33 nM and τ values were 4, 9, 38, at hOTR, mOTR, hV1aR, respectively. OT activity was 25-fold higher than AVP ($\Delta\text{Log}[\tau/K_A] = 1.40 \pm 0.04$, n=9, $P<0.05$) at hOTR, and 5-fold higher than AVP ($\Delta\text{Log}[\tau/K_A] = 0.74 \pm 0.21$, n=3, $P<0.05$) at mOTR. AVP activity was 235-fold higher than OT at hV1aR ($\Delta\text{Log}[\tau/K_A] = 2.37 \pm 0.04$, n=7, $P<0.05$).

Discussion: We demonstrate an innovative approach for controlling receptor number which allows researchers to access the operational model without an irreversible antagonist. In terms of IP1 accumulation, there seems to be a greater possibility for cross-talk at mOTR than hOTR. This approach may provide a useful tool for uncovering the pharmacodynamic consequences of receptor mutations, species differences and defining biased signalling or scaling drug activity, all of which are crucial for rational drug discovery.

Song Z and Albers H (2018). Frontiers in Neuroendocrinology, 51, pp.14-24.

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Targeting spinal A1 adenosine receptors with allosteric modulators to reduce pain signalling

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Introduction: In response to noxious stimuli, nociceptive signals are transmitted from the periphery to the spinal cord dorsal horn and then to the brain, where the signals are perceived as pain. The afferent nerves that carry these signals to the spinal cord express adenosine A1 receptors (A_1ARs) which are Gi-coupled G-protein coupled receptors (GPCRs). When A_1ARs are activated by their orthosteric agonist adenosine, activity of the neuron is decreased, reducing nociceptive signalling. The activity of orthosteric agonists can be modulated by activation of the allosteric site of the receptor.

Aims. This study examined the effects of a novel positive allosteric modulator (PAM) MIPS521 [(2-amino-4-(3,5-bis(trifluoromethyl)phenyl)thiophen-3-yl)(4-chlorophenyl) methanone] on intrinsic neuronal activity at spinal cord dorsal horn neurons in a rat model of neuropathic pain.

Methods: Whole cell patch-clamp electrophysiology was used to investigate activity of specific spinal neurons. The rat model of neuropathic pain used was partial nerve ligation (PNL) of the sciatic nerve.

Results and Discussion: MIPS521 was found to be specific for the A1 subtype of adenosine receptor, as A2B and A2A antagonists produced additional hyperpolarisation in the presence of MIPS521. It was theorised that the inhibitory mechanism of MIPS521 involves increased potassium (K+) conductance, as the activity of MIPS521 on lamina II excitatory neurons was reversed by K+ channel blockers. MIPS521 was also shown to be effective across species, as it reduced neuronal activity in *ex vivo* preparations from the macaque dorsal horn. The effects of T62, an older generation A1AR PAM, were compared with MIPS521. T62 showed similar, but lower levels of activity. It is proposed that the allosteric site of the A1AR is a potential therapeutic target for neuropathic pain, as the data shows that MIPS521 reduces nociceptive activity in spinal pain pathways.

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The orexins exhibit distinct pharmacological profiles at orexin receptor 2

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Introduction: The orexins, orexin A (OxA) and orexin B (OxB), are peptide agonists that bind to orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2). OxA binds to both receptors with similar affinity while OxB exhibits substantially decreased affinity for OxR1. OxR2 has been reported to couple to G_q and G_i class G proteins in the CNS, however, the effect of each orexin on G protein activation and other aspects of OxR2 pharmacology is yet to be determined.

Aims. We aimed to investigate aspects of the orexins' pharmacology at OxR2 that to our knowledge have not been elucidated to date.

Methods: We achieved this utilizing cutting-edge bioluminescence resonance energy transfer (BRET) technologies to monitor protein-protein proximity within live HEK293FT cells in real time.

Results: We found that OxA and OxB exhibit different potencies in their stimulation of $G\alpha_q$ and $G\alpha_i$ subunit disassociation from $G_{\gamma 2}$. The potency of Gα subunit disassociation is G protein specific and exhibits time-dependent effects. Following OxR2 stimulation, OxB treatment results in an approximate 2.5-fold increase in BRET signal indicative of β-arrestin2 recruitment to the plasma membrane over OxA treatment. However, while OxA treatment leads to the establishment of a slowly decaying plateau in β-arrestin2 recruitment over 2 hours, OxB-induced β-arrestin2 recruitment exhibits a comparatively rapid decrease in plasma membrane proximity, with recruitment becoming non-significantly different to OxA treatment at approximately 50 minutes post-ligand administration ($n=3$, $P<0.05$). Moving away from the plasma membrane, OxB treatment induces a significant increase in OxR2 trafficking to Rab11a-containing endosomes (recycling endosomes) over OxA treatment ($n=3$, $P<0.05$).

Discussion: These findings demonstrate that the orexins induce distinct pharmacological responses upon activation of OxR2 in HEK293FT cells, with critical signal transduction machinery exhibiting distinct, ligand-dependent interactions. This has implications for our understanding of hypothalamic neuromodulatory pathways and orexin-targeted drug development.

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The role of caveolae in osmotic pressure-induced glioblastoma invasiveness

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Introduction: In solid tumours, elevated interstitial fluid pressure (osmotic and hydrostatic pressure) is a barrier to drug delivery, but also correlates with poor prognosis. Glioblastoma (GBM) experience compressive force when growing within a space limited by the skull. Caveolae are proposed to play mechanosensing roles and caveola-forming proteins are overexpressed in GBM. We asked whether caveolae mediate GBM response to osmotic pressure.

Aims. To evaluate *in vitro* the influence of spontaneous or experimental downregulation of caveola-forming proteins (caveolin-1, CAVIN1) on the proteolytic profile and invasiveness of GBM cells in response to osmotic pressure.

Methods: We employed GBM cell lines which spontaneously express (U87, U251) or lack (U118) caveola-forming proteins. We silenced caveolin-1 or CAVIN1 by siRNA, shRNA or CRISPR-Cas9. The effect of osmotic pressure on the proteolytic profile and epithelial–mesenchymal transition (EMT) was investigated using zymography and real-time qPCR. Invasion through basement membrane-like protein was assayed *in vitro*.

Results: GBM cell lines expressing caveola-forming proteins upregulated plasminogen activator (uPA) and/or matrix metalloproteinases (MMPs), some EMT markers and increased *in vitro* invasion potential in response to osmotic pressure. Downregulation of caveola-forming proteins impaired this response.

Discussion: GBM respond to osmotic pressure by increasing matrix degrading enzyme production, mesenchymal phenotype and invasion. Caveola-forming proteins mediate, at least in part, the proinvasive response of GBM to osmotic pressure. This may represent a novel target in GBM treatment.

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Brain activity in response to z-drugs: role for $GABA_A$ γ1 and γ3 subunits

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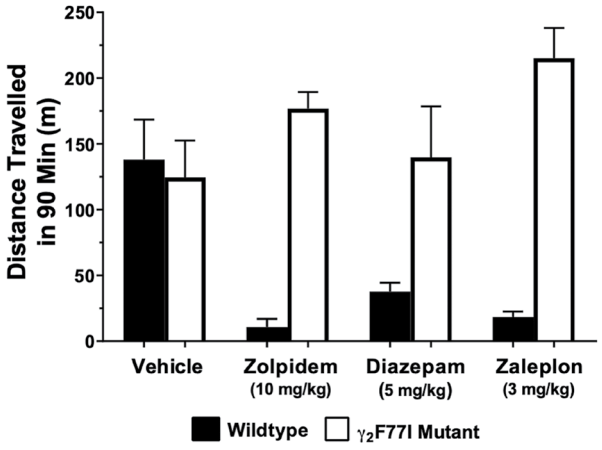
Introduction: Z-drugs bind to $GABA_A$ receptors at the interface between α_x-γ_2 subunits. However, many have interactions with γ1 and γ3 subunits, that are expressed throughout the striatum and globus pallidus[1,2]. Little is known about the physiological importance of these receptors within these regions.

Aims. We used the γ_2F77I[3] knock-in mouse which has drastically attenuated z-drug binding to γ_2-$GABA_A$ receptors to determine any residual z-drug effects via γ1 and γ3 subunits.

Methods: Using c-Fos immunohistochemistry we investigated responses from zolpidem (10 mg/kg), diazepam (5 mg/kg), zaleplon (3 mg/kg), and vehicle in 12-week-old male γ_2F77I mice and wildtype littermate controls across 40 brain regions. Additionally, locomotor activity was tracked in an open field test to determine sedation (Figure).



Results: In the open field test, wildtype mice treated with diazepam and z-drugs had significantly less activity than mutant mice (two-way ANOVA ($F(7, 28) = 13.45$, $p<0.01$, $n=5$). The c-Fos study showed that in in wildtype mice, zolpidem and diazepam showed less c-Fos positive cells in the striatum, (16±7; 13±3) compared to vehicle, (173±56, $n=4$, $p<0.05$) and similarly the globus pallidus (5±3, 1±1) also had less activation compared to controls (34±12 $n=3$, $p<0.05$). The γ2F77I mice showed no significant differences compared to vehicle control in these regions ($n=7$).

Discussion: The globus pallidus and striatum are both associated with generation of movement. This study demonstrates that γ_2F77I mice are unaffected by z-drugs and diazepam in these regions while wildtype mice have a suppression of activity.

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Investigation of the specificity of cannabidiol signalling

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Introduction: The use of the non-psychoactive phytocannabinoid cannabidiol (CBD) as antiepileptic and antipsychotic has been rapidly increasing, however the molecular mechanisms through which CBD may exert its therapeutic actions has not been assessed rigorously, and few in vitro studies have demonstrated acute inhibitory effects on CB1 signaling.

Aims. In order to gain a deeper understanding of the pharmacological activity of CBD at cannabinoid and non-cannabinoid receptors, we examined the real-time signalling of multiple receptors in the presence of CBD.

Methods: AtT-20 and HEK293A cells expressing either human cannabinoid receptors CB1, CB2, μ-opioid receptors (MOR), or dopamine receptors (D2R) were pre-treated with 100 nM or 10 μM CBD prior to the subsequent addition of CP55940 (cannabinoid agonist), morphine (MOR agonist), or quinpirole (D2R agonist) at 37 °C or at room temperature (RT, 24 °C) (to mimic some recent studies of CBD). We measured real-time activation of potassium channel-mediated cellular hyperpolarisation and accumulation of cellular cAMP levels using a BRET biosensor (CAMYEL).

Results: Acute application of CBD for 5-15 mins at 24 °C significantly reduced the agonist-induced hyperpolarisation of CB1, CB2, or MOR compared to the vehicle treated cells, whereas no effect was observed for experiments at 37 °C. Consistent with this, CBD negatively modulated inhibition of cAMP accumulation by CB1 and D2R activation at 24 °C but not 37 °C. We next examine the prolonged effects of CBD on these receptors. CBD (10 μM, 60 mins) significantly reduced the maximal response of CP55940 induced hyperpolarisation of CB1 (Control, 33 ± 1%; with CBD 10 μM 18 ± 1%) at 37 °C, but failed to significantly affect the agonist-induced hyperpolarisation in AtT20 cells expressing CB2 or MOR ($P > 0.05$).

Discussion: Our data suggests that the promiscuity of CBD interaction with multiple receptors is temperature dependent. However, our study, confirms the selective inhibitory effects of CBD on CB1 responses. The unspecific effects of CBD at room temperature highlight the value of assays with physiological face validity, and also re-inforce the need to include a range of receptors.

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Rational Replicons: A Viral Replication Model for Memory Engrams

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Introduction: Similarities between engineered and natural control systems have been evident for decades, as has the clear superiority of the latter, which have been refined by billions of years of evolution. The smallest known viruses of vertebrates, the deltaviruses, exemplify primitive biological control systems: these tiny single stranded circular RNA (cRNA) replicons can respond to changes in their immediate environment in a variety of ways that maximise their survival chances, a "cognitive" ability sometimes regarded as life-defining. Endogenous cRNAs, which are increasingly being recognised as essential regulators of gene expression in all living cells, are particularly abundant in neural tissues and concentrated at synapses. All neurons express genes for amyloid-like RNA-binding proteins that regulate transcription and translation of RNAs that are essential neural plasticity: amongst this group, mammalian CREB3 genes are unique because they encode a deltavirus-type ribozyme processes its transcripts. A rare single nucleotide polymorphism that increases CREB3 ribozyme efficiency impairs short-term memory. CREB3 transcripts include a host of cRNAs, at least two of which contain intact ribozymes, so may be considered deltavirus analogues.

Aims. To examine the deltaviral survival strategy from a control systems perspective, in order to gain more insight into the analogous neural CREB3 RNA processing system, and *vice versa.* To eventually generate biochemical-genetic, mathematical and electronic models of CREB3 and deltavirus control networks and test their predictive capacity.

Methods: Gene sequences and related information were extracted from NCBI and analysed using a variety of freely accessible internet resources. The deltavirus replication and survival strategy was abstracted as a network of interactive subsystems consistent with the concepts of self-organising criticality and highly optimised tolerance.

Results: Deltavirus survival, (and by extension, memory retention) depends on the ability to rationalise resource use so as to simultaneously maintain two sets of information that differ in their relatively stability, size and accuracy.

Discussion: Essentially similar systems control information acquisition, modification and storage stability in populations of deltaviruses and animal neurons.

Shobana S Liang WS (2019) Non-coding RNA Research 4:23-29.

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Divergent pathways mediate the effects of a 5-HT1A receptor agonist on close social interaction, grooming and aggressive behaviour in mice: exploring involvement of the oxytocin system

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Introduction: 5-HT_{1A} receptor (5-HT_{1A}R) abnormalities are implicated in aggression and there has been considerable interest in developing 5-HT_{1A}R agonists for treating aggression. Endogenous oxytocin released upon stimulation of 5-HT1ARs in the hypothalamus mediates at least some of the effects of 5-HT_{1A}R agonists on social behaviour.

Aims. Given 5-HT_{1A}R, oxytocin receptor (OXTR) and vasopressin V1a receptor (V1aR) agonists can all reduce aggression, the current study aimed to determine whether the anti-aggressive effects of 5-HT_{1A}R stimulation can also be explained by downstream actions at OXTRs and/or V1aRs in a mouse model of non-territorial, hyper-aggressive behaviour.

Methods: Male Swiss mice (N=80) were socially isolated or group housed for 6 weeks prior to the start of testing. Testing involved placing two unfamiliar weight and condition matched mice together in a neutral context for 10 min.

Results: Social isolation led to a pronounced increase in aggressive behaviour, which was dose-dependently inhibited by the 5-HT1AR agonist 8-OH-DPAT (0.1, 0.3, 1 mg/kg i.p.) with accompanying increases in close social contact (huddling) and grooming. The effects of 8-OH-DPAT on aggression, huddling and grooming were blocked by pre-treatment with a selective 5-HT_{1A}R antagonist (WAY-100635, 0.1 mg/kg i.p.). The anti-aggressive effects of 8-OH-DPAT were unaffected by an OXTR antagonist (L-368,899, 10 mg/kg i.p.), whereas the effects on huddling and grooming were inhibited. Pre-treatment with a V1aR antagonist (SR49059, 20 mg/kg i.p.) had no effect.

Discussion: Our study suggests that stimulation of endogenous oxytocin is involved in the effects of 5-HT_{1A}R activation on close social contact and grooming but not aggression.

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Assessing assay bias in fluorescence-based high throughput screening of voltage-gated sodium channels

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Introduction: Voltage-gated sodium channels (NaVs) are a major target for the development of therapeutics in epilepsy, pain and cardiac conditions. High throughput fluorescence-based screening assays are used increasingly to screen for novel NaV modulators, particularly in the venoms-to-drugs field. However, results obtained from fluorescence-based assays do not always correlate well with results obtained from conventional patch-clamp electrophysiology and NaV channel pore-blocking modulators are favoured over gating-modifiers.

Aims. Identify the causes of assay bias in fluorescence-based assays using different NaV channel modulators and develop novel strategies to perform unbiased high-throughput screening of Na_v channel modulators.

Methods: HEK293 and CHO cells expressing human NaV1.1-1.8 (SB Drug Discovery; Chantest) were used in functional assays. The FLIPR^{TETRA} system (Molecular Devices) was used in fluorescence-based multi-plate format assays with either membrane potential dye (Molecular Devices) or the Asante NaTRIUM Green-2 AM sodium indicator dye (Abcam). Whole-cell patch clamp electrophysiology experiments were performed using the QPatch-16 automated patch-clamp platform (Sophion Bioscience).

Results: Fluorescence-based assays successfully identified NaV modulators. The EC50 values of NaV activators obtained in the FLIPR correlate well with those obtained by electrophysiology. However, NaV inhibitor screening results in assay bias as pore-blocking compounds (e.g. tetrodotoxin, tetracaine) have IC_{50} values that correlate with electrophysiology, but gating modifiers (e.g. μ-theraphotoxin-pn3a) did not. The use of veratridine at increasing concentrations to activate channels in NaV inhibitor screens caused decreased potency. Application of veratridine to whole-cell patch clamp produced corresponding shifts in potency. The use of alternative NaV activators (e.g. brevetoxin and α-scorpion toxin OD1) also results in similar shifts in potency.

Discussion: NaV channels are activated by voltage *in vivo*, but fluorescence-based cell assays in heterologous expression systems require the use of allosteric activators. The binding of these activators likely promotes an unfavourable channel state or compete with inhibitors for binding. This results in the preferential detection of pore blockers over gating modifiers.

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A prognostic model for advanced lung cancer patients treated with atezolizumab

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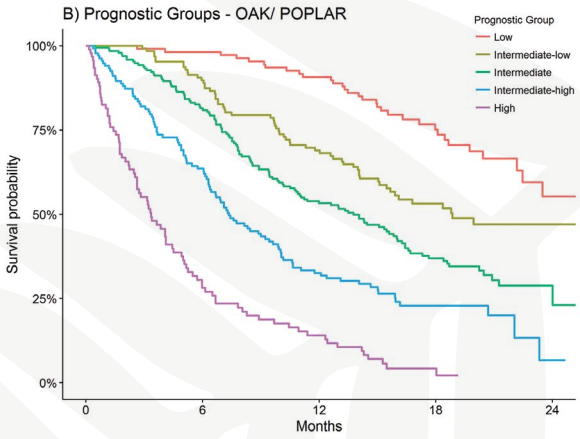
Introduction: Immune checkpoint inhibitors (ICI) are a significant advance to non-small cell lung cancer (NSCLC) treatment, however, outcomes are still associated with significant heterogeneity. This study aimed to develop and validate a pre-treatment prognostic tool of survival in NSCLC patients treated with ICIs.

Methods: Decision-tree, Cox proportional hazard and random forest analysis were evaluated to determine a prognostic model using clinicopathological data. Development data consisted of advanced NSCLC patients treated with atezolizumab from the randomized trials OAK and POPLAR (n=751). Data from the single-arm atezolizumab trials BIRCH and FIR (n=797) were used for external validation. Prognostic groups were defined as the lower 15th (low), 15th-35th, 35th-65th, 65th-85th, and upper 15th (high) risk percentiles. The primary outcome was overall survival (OS), with progression-free survival (PFS) secondary.



Results: Pre-treatment C-reactive protein (CRP) was the single most predictive variable for OS. The prognostic tool was defined by CRP, lactate dehydrogenase, derived neutrophil-to-lymphocyte ratio, albumin, PD-L1 expression, performance status, time since metastatic diagnosis, and metastatic sites count. Identified prognostic groups had significantly different OS probabilities (P<0.001), with median OS ranging from 3 to >24 months for the high to low risk group within OAK and POPLAR. Similar findings were identified for PFS (P<0.001), with median PFS ranging from 1 to 5 months for the high to low risk group.

Conclusions: A prognostic tool was developed and validated to identify patient groups with distinctly different survival probabilities following atezolizumab initiation for advanced NSCLC.

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Risk of Falling and the Use of Falls Risk Medications in Residents of Aged Care Services

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Introduction: Falls are one of the most common causes of unintentional injury-related fatalities worldwide, with people aged 65 years or older at the highest risk. Ceasing or reducing the dose of falls-risk-increasing drugs (FRIDs) is a potentially important strategy to prevent falls in aged care services.

Aims. To investigate the association between the risk of falling and use of FRIDs in residents of aged care services.

Methods: Inverse probability weighted multinomial logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between falls risk and regular use of FRIDs in 383 residents from six Australian aged care services. FRIDs include psychotropic medications and medications that can cause or worsen orthostatic hypotension (OHs).

Results: Overall, the prevalence of FRIDs was similar between residents of high (91.8%) and low (92.3%) falls risk. The most frequently used FRIDs were antidepressants (47.8%), diuretics (43.3%) and renin-angiotensin system inhibitors (43.1%). The prevalence of antipsychotics (9.1%) and sedative-hypnotics (14.1%) was low. Residents at high falls risk had higher adjusted odds of using ≥2 psychotropic medications (OR=1.75, 95%CI 1.17-2.61) and ≥2 OHs (OR=3.59, 95%CI 2.27-5.69).

Conclusion. Residents at high falls risk had higher odds of using ≥2 FRIDs than residents at low falls risk. High prevalence of FRIDs was mainly attributable to medications for which residents had clinical indications, such as antidepressant medications or cardiovascular medications. Clinicians appeared to have largely avoided FRIDs that explicit criteria deem potentially inappropriate for high falls risk.

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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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Caregivers' experiences of medication management advice for people with dementia at hospital discharge: A qualitative study

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Introduction: The Australian Commission on Safety and Quality in Health Care in report in 2014, has highlighted a major gap in post-discharge services provided to people with dementia and their caregivers. Caregivers of people with dementia play an important role in managing medications across transition of care and need to be provided clear guidance on medication management for people with dementia on discharge. However, at present, it is unclear whether this occurs in routine clinical care.

Aims. To explore caregivers' experiences and perspectives towards medication management guidance and resources provided at hospital discharge.

Methods: One-on-one semi-structured interviews will be conducted with caregivers of people with dementia. Our inclusion criteria will be: 1) caregivers who are providing care for a person with dementia discharged from the hospital in the last 12 months; and 2) care recipients living in the community or reside in aged care homes. Purposive sampling will be used to ensure maximum variation to ensure diverse experiences and perspectives are covered. We will recruit first in NSW followed by other major states until saturation is reached with an estimated target sample of 40 participants. Analysis of the interviews will be carried out using an inductive thematic approach to identify patterns and derive meaning within the dataset to answer the aims of the study.

Results: At this stage, we have obtained ethics approval and are currently in the process of recruiting potential participants. Preliminary results will be available at the conference.

Discussion: This study will generate insights into what support caregivers' need at discharge to standardize the management of medications of people living with dementia which may lead to better quality care. The findings will also be to inform the development of a national survey for caregivers.

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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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Viral infection exacerbates cigarette smoke-induced endothelial dysfunction in the mouse thoracic aorta.

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Introduction: Chronic obstructive pulmonary disease (COPD) is a debilitating disease characterised by persistent airflow limitation, lung inflammation and overexuberant oxidative stress. COPD is currently the 4th leading cause of death globally, with cigarette smoking (CS) being the largest causative factor of this disease. Approximately 50% of all COPD patients will die as a direct result of cardiovascular disease. However, patients often experience episodes of worsened symptoms known as acute exacerbations of COPD (AECOPD) caused by viral or bacterial infection, further increasing the likelihood of mortality. Although increased oxidative stress alters blood vessel structure through promoting vascular remodeling, stiffening and atherosclerosis, the mechanism underlying these worsened cardiovascular outcomes during AECOPD remains unknown.

Aim: To investigate the effect of viral infection on vascular endothelial function in mice exposed to CS.

Methods: Male BALB/c mice were exposed to either room air (sham) or CS generated from 9 cigarettes per day, 5 days a week for 8 weeks followed by intranasal inoculation with influenza A virus (Mem71, 1 x $10^{4.5}$ pfu). Mice were culled at days 3 and 10 post-infection. Upon sacrifice, the thoracic aorta was removed, cut into ~2 mm rings, mounted in a myograph and set to a resting tone of 5mN of force. The aortic rings were pre-constricted to their maximum tension using the thromboxane A2 receptor agonist U46619 (100nM), then washed with Krebs and pre-constricted to 50% maximum using U46619. Concentration-response curves to acetylcholine (Ach) or sodium nitroprusside (SNP) were then performed to investigate endothelial and smooth muscle dilator responses, respectively.

Results: Ach (10^{-8} to 10^{-5} Mol/L) caused an ~85% relaxation of U46619-contracted aorta obtained from sham-exposed mice irrespective of viral infection (n=8). However, aorta from CS-exposed mice had significantly impaired relaxant responses to Ach (n=8, ~50 % R_{max}, $p<0.0001$), which was further impaired following Mem71 infection (n=8, ~35% R_{max}, $p<0.01$). SNP (10^{-8} to 10^{-5} Mol/L) caused an ~90% maximum relaxation of aorta taken from both sham and CS-exposed mice, irrespective of viral infection.

Discussion: Endothelial function of the thoracic aorta is significantly impaired following CS exposure and further worsened following viral infection.

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Effects of prolonged exposure to paracetamol on late gestation rat placenta

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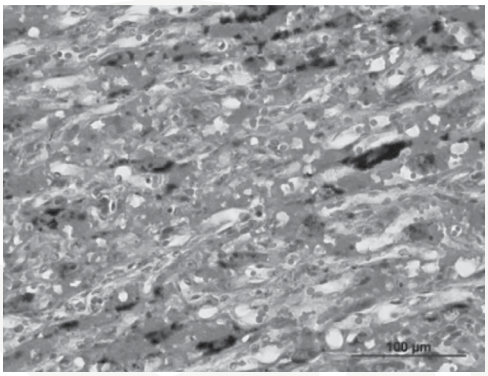
Introduction: Paracetamol (acetaminophen) is one of the most commonly used drugs and over 70% of women take it during pregnancy (Werler et al., 2005). However, its ability to cross the placenta and potential toxicity in placenta and fetuses is unknown.



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 ([3H]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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TLR4-dependent and -independent immune activation by cancer cell lysates

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Introduction: Damage associated molecular patterns (DAMPs) are released by cancer cells in response to cell stress, damage or death. DAMPs stimulate the immune system through interacting with a broad range of receptors including Toll-like receptors, thereby triggering both anti-tumor and tumor-promoting pathways.

Aims. To test the modulation of TLR4 signaling elicited by DAMPs produced by cancer cells.

Methods: TLR4-activation was tested using HEK-Blue™ hTLR4 reporter cells (engineered to produce secreted embryonic alkaline phosphatase (SEAP) via NF-κB-mediated activation) together with their control cell lines, HEK-Blue™ Null2 cells and HEK-Blue™ hMD2-CD14 cells. Cell lysates were generated by freeze-thawing MDA-MB-231 and MDA-MB-468 breast cancer cells. HEK-Blue cells were treated with different concentrations of cell lysates, in the presence or absence of the TLR4 antagonist, LPS-RS.

Results: Cell lysates from breast cancer cells demonstrated a concentration-dependent induction of the reporter gene in HEK-Blue™ hTLR4 cells, but also in the control cells which do not overexpress TLR4. The TLR4 antagonist, LPS-RS (100 ng/ml), significantly but incompletely (up to 50%) inhibited the activation of HEK-Blue™ hTLR4 cells by cell lysates. In contrast, up to 86% inhibition was achieved by LPS-RS on activation induced by the TLR4 agonist, LPS.

Discussion: DAMPS produced by cancer cells during active cancer directed treatment may activate immune cells via both TLR4-dependent and independent pathways.

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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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Glycolysis modulates NADPH oxidase 2 activity through the pentose phosphate pathway and Type I interferon-β

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Introduction: Pathogenic macrophages undergo a metabolic switch from oxidative phosphorylation to glycolysis, which occurs during sepsis induced by LPS, as well as cancer. This causes a sustained inflammatory phenotype whereby reactive oxygen species (ROS) and inflammatory cytokines are overproduced. It remains unknown whether this inflammatory profile is a product of the metabolic switch that occurs upon activation of macrophages.

Methods: RAW 264.7 macrophages were treated with 2-deoxy-d-glucose (2DG; glycolysis inhibitor; 1mM), dimethyl succinate (cell-permeable exogenous succinate; 5mM), buthionine sulfoximine (glutathione inhibitor; 50μM), mitoTEMPO (mitochondrial ROS scavenger; 1mM) or 6-aminonicotinamide (6AN; pentose phosphate pathway (PPP) inhibitor; 200μM), and stimulated with LPS (100ng/ml) or IFN-β (100ng/ml) for 24 hours. Cells were analysed for gene expression, protein quantification, oxidative burst and glutathione quantification. For *in vivo* studies, male C57BL/6 mice (8-12-weeks old) were treated with 6AN (10mg/kg; i.n.) daily starting one day prior to LPS exposure (10μg/mouse; i.n.). Mice were culled one day post LPS exposure for assessment of airway inflammation.

Results: Inhibition of glycolysis or PPP significantly suppresses the capacity of LPS to induce an oxidative burst response via the NOX2 oxidase enzyme in macrophages by ~30%, associated with a significant reduction in the gene expression of NOX2 and its organiser $p47^{phox}$ ($p<0.001$, n=8-9). LPS-dependent enhancement in NOX2 oxidase activity was independent of both succinate and mitochondrial ROS production. LPS also increased Type I IFN-β expression and protein, which was completely suppressed by inhibitors of both glycolysis and the PPP. Moreover, recombinant IFN-β increased NOX2 oxidase dependent ROS production, as well as NOX2 and p47phox expression by ~45% ($p<0.05$, n=7-8). When 6AN was administered i.n., we observed ~50% reductions in LPS-induced infiltration of macrophages, neutrophils and lymphocytes ($p<0.01$, n=6-8).

Discussion: Our findings identify previously undescribed molecular mechanisms by which both glycolysis and the PPP modulate inflammation. We propose that glycolysis and the PPP initiate an intricate feedback loop that involves NOX2 and IFN-β, which controls oxidative stress and antiviral Type I IFNs, and thereby inflammation.

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Living Fast and Dying Young: Guanine Oxidation and Mitochondrial Genomic GC Content as Regulatory Factors

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Introduction: Both age-related mitochondrial dysfunction and telomere attrition are recognised as important contributors to cell senescence and ageing. At the molecular level, superoxide (SO) produced in response to pro-inflammatory stimuli and as a by-product of respiration is the main precursor of potentially damaging reactive oxygen species (ROS) for which guanine (G), as the nucleobase most susceptible to oxidation, is a prime target, 8-oxoguanine (OG) being the major product. During prolonged stress, the availability of un-oxidised G nucleotides may become critical for cell survival. Mitochondrial genomes are extremely susceptible oxidative damage due to their proximity to sites of ROS generation. Mitochondrial genomes of vertebrates have highly asymmetric base composition, with heavy (H) and light (L) strands, due to high G and C content respectively. Intriguingly, - considering that they encode only a single peptide -, full-length G-rich transcripts of the L strand are produced and degraded faster than H strand transcripts, implying that this energetically wasteful process probably serves some biological function(s).

Aims. To investigate the relationships between the mitochondrial genomic GC content and key life history variables in representative primate and rodent species for which appropriate and reliable data was available.

Methods: Complete mitochondrial genomic reference sequences were extracted from GenBank. Anatomical, physiological and life history data was obtained from publications available via NCBI PubMed. Bivariate allometric analyses were performed on untransformed data using TableCurve2D statistics-graphics software.

Results: Mitochondrial GC content has tended to increase in parallel with strand bias (G-skewness) during evolution of both rodents and primates. Positive allometric relationships between the GC of mitochondria and key life-history variables including body mass, maximum lifespan and maximal respiratory capacity were observed.

Discussion: Primates and large mammals live longer than smaller mammals which are (with notable exceptions) more prone to cancer (Peto's paradox). Replicative senescence triggered by telomere attrition is one mechanism by which this is achieved. Our observations suggest that accumulating a mitochondrial G nucleotide reserve may be another, and this has some important implications, particularly for medical research that uses rodent models.

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Therapeutic targeting of endosome and mitochondrial ROS protects mice from influenza infection

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Introduction: Influenza viruses are highly infectious respiratory pathogens that cause widespread epidemics and presents the potential for major pandemic outbreaks. The emergence of antiviral resistance dictates a need for novel therapeutic approaches that target viral pathology irrespective of the infecting strain. Influenza viruses drive the production of reactive oxygen species (ROS) that suppress anti-viral responses and contributes to pathological lung inflammation and morbidity. The major sites of ROS are endosomes via the NOX2-oxidase enzyme and the electron transport chain of the mitochondria.

Aim: To examine the effect of therapeutic administration of an endosome-targeted NOX2 oxidase inhibitor (Cgp91ds-TAT) in combination with a mitochondrial-targeted ROS scavenger (mitoTEMPO) against influenza A virus (IAV) infection.

Methods: Male C57Bl/6 mice were infected with IAV (Hong Kong X-31 strain; 10^4 PFU/mouse) and treated once daily with Cgp91ds-TAT (0.2mg/kg) and mitoTEMPO (100µg) intranasally from one day post-infection (p.i) up to 6 days p.i. Mice were euthanized at 3 and 6 days p.i for analysis of lung inflammation, oxidative stress and viral burden.

Results: The combination of Cgp91ds-TAT and mitoTEMPO resulted in a substantial reduction in airway neutrophilic inflammation, viral load and bodyweight loss induced by IAV. Combination therapy also improved the lung pathology as characterised by a reduction in both alveolitis and peri-bronchiolitis. At the early stages of infection (day 3 p.i), there was a significant elevation of Type I IFN expression and IL-1β expression that was suppressed at the later phase of infection (day 6 p.i). Furthermore, dual inhibition of ROS with Cgp91ds-TAT and mitoTEMPO caused elevations in activated B cell populations but did not alter local T cell immunity.

Discussion: There is an urgent need to develop novel therapeutic strategies against IAV infections given the emergence of antiviral resistance and lack of vaccine efficacy. This is the first study to demonstrate that effective reduction of ROS production in the two major subcellular sites: the endosome and mitochondria, by intranasal supplementation of Cgp91ds-TAT and mitoTEMPO is a potential host-targeted therapeutic approach to IAV infection.

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CYP2D6 genotype distribution in Aboriginal and non-Aboriginal Australians

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Introduction: Genetics can play a major role in inter- and intra-ethnic differences in drug response and toxicity; the drug metabolising enzyme gene *CYP2D6* is a major and clinically-actionable example. *CYP2D6* genetic variability results in poor (PM), intermediate (IM), normal (NM) and ultrarapid (UM) metaboliser phenotypes which vary in frequency between ethnicities, with some prescribing guidelines recommending pre-emptive *CYP2D6* genotyping for different medicines. Relatively little is known of *CYP2D6* genotype distribution in Aboriginal Australians (AA), although Griese et al (2001) reported a low frequency of then known variant *CYP2D6* genotypes in a remote AA population of North-Western Australia.

Aims. To determine the *CYP2D6* genotype distributions of AA and non-Aboriginal Caucasian (NA) populations of Southern Australia.

Methods: One hundred and thirty-seven AA and 159 NA provided saliva samples for DNA isolation and genotyping of 31 *CYP2D6* single nucleotide polymorphisms and small insertions/deletions (Affymetrix DMET Plus Array) as well as gene copy number variation (CNV) and hybrid allele detection (Agena Veridose). Genotypes were translated into predicted metaboliser phenotypes (Crews et al., 2014) and frequencies compared between AA and NA by Chi^2 tests.

Results: Forty-five different *CYP2D6* genotypes were detected. Hybrid alleles were detected in 7% of AA and 9% of NA, but this did not alter their predicted phenotypes. Heterozygous deletions were detected in 8% of AA and 5% of NA, and functional gene multiplications were detected in 3% of AA and 1% of NA. CNV detection changed the predicted phenotypes (vs without CNV detection) in 2% each of AA and NA. The distribution of common (overall frequency >4%) genotypes differed significantly between AA and NA (P=0.007). UM, NM, IM and PM phenotype frequencies were 1.5%, 93%, 4.4% and 1.5% in AA and 0.6%, 85%, 7% and 7.6% in NA, respectively (P=0.05).

Discussion: This confirms *CYP2D6* genetic variability differs between AA and NA, and that AA are predominantly CYP2D6 NM. Whether this translates to better response to substrate prodrugs, or reduced toxicity, is unknown.

Crews K et al (2014) Clin Pharmacol Ther 95:376-82.
Griese UE et al (2001) Pharmacogenet 11:69-76.

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Prediction of pharmacokinetics in lornoxicam by CYP2C9 genetic polymorphism: from in vitro to clinical studies

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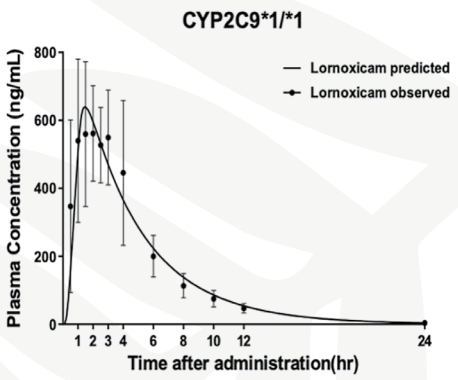
Introduction: Lornoxicam, a non-steroidal anti-inflammatory drugs (NSAIDs) of oxicam class with analgesic and anti-inflammatory effects, is used in rheumatoid arthritis and osteoarthritis patients. Lornoxicam is completely metabolized to 5'-hydroxy lornoxicam, mediated by cytochrome P450 2C9 (CYP2C9). *CYP2C9*3* and *CYP2C9*13*, found in East Asian population, are reported to be associated with decreased CYP2C9 enzyme activity. Therefore, difference of CYP2C9 enzyme activity considerably affects pharmacokinetics of lornoxicam.



Aims. We focus development of predictable pharmacokinetic model based on physiological properties according to CYP2C9 allele enzyme activities.

Methods: 13 healthy Korean volunteers were participated in pharmacokinetic study of lornoxicam. All subjects were divided into three group according to CYP2C9 genotypes *CYP2C9*1/*1, CYP2C9*1/*3, CYP2C9*1/*13*. Each subject received an oral dose of 8 mg lornoxicam. After administration, venous blood samples were collected and analyzed by HPLC-UV method. PBPK software PK-Sim®8 (Bayer AG, Germany) was used to predict pharmacokinetic parameter according to CYP2C9 enzyme subtypes. For model development and validation, simulated pharmacokinetic data were compared to our clinical study data using suitable assessment 99.998% confidence interval criteria and two-fold criteria. For more accuracy, liver standard volume had altered.

Results: In our research, $AUC_{0\text{-}24}$, AUC_{inf}, C_{max}, T_{max}, and clearance (CL/F) were dramatically changed in *CYP2C9*1/*3* and *CYP2C9*1/*13* compared to *CYP2C9*1/*1* genotype. These parameters were simulated, Simulation data in every groups were fit suitable criteria. Pharmacokinetic studies of lornoxicam for validation were fit likewise.

Discussion: PBPK model of lornoxicam was successfully developed. However, since there was no pharmacokinetic profile data of white and black people, we only conducted validation about Chinese ethnic. Therefore, we expect to be able to create more accurate model with pharmacokinetic data of these ethnic.

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Estimating concentration of venlafaxine and its metabolite in case of enzyme polymorphism and renal impairment

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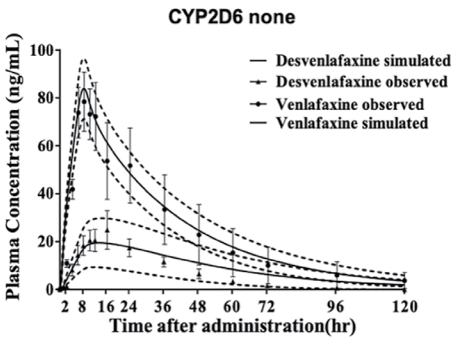
Introduction: Venlafaxine (VEN) is serotonin-norepinephrine reuptake inhibitor (SNRI) class used in depression and anxiety patients. VEN is mostly metabolized to desvenlafaxine, mediated by cytochrome P450 2D6 (CYP2D6). VEN is also metabolized to N-desmethyl venlafaxine (NDV) slightly and desvenlafaxine is metabolized to N, O-didesmethyl venlafaxine (NODV). Several variant polymorphism of CYP2D6 gene change its enzyme activity, e.g., decreased activity by *CYP2D6*10* and abolished activity by *CYP2D6*5*. Therefore, difference of *CYP2D6* alleles affects pharmacokinetics of venlafaxine and its metabolite. Renal function also affects pharmacokinetics of venlafaxine and its metabolite because both compounds are dominantly excreted by kidney.



Aims. We focus development of predictable model based on various physiological properties such as CYP2D6 allele enzyme activities and renal function.

Methods: We did comprehensive search of literature and collected pharmacokinetic profile data of venlafaxine and desvenlafaxine. We extracted time-concentration profile value by converting from visual data to numerical data using Engauge Digitizer®11.3. PBPK software PK-Sim® 8 (Bayer AG, Germany) was used to predict pharmacokinetic parameter according to CYP2D6 genetic polymorphism and renal impairment. Observed pharmacokinetic data were compared to simulated data using appropriate assessment e.g., 99.998% confidence interval or two-fold criteria.

Results: Pharmacokinetic data such as $AUC_{0\text{-}24}$, AUC_{inf}, C_{max}, T_{max} had considerably changed in CYP2D6 genetic polymorphism and renal impairment. Every value for model development and validiation adequately simulated, included in suitable criteria. Therefore, venlafaxine and its metabolite PBPK modeling was successfully developed.

Discussion: For more accurate model, we need more pharmacokinetic profile of compounds in renal impairment situation. Also, further studies need to consider minor metabolism of venlafaxine and its metabolite specifically.

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Simulation of metoclopramide pharmacokinetic model according to CYP2D6 genotypes in adults and pediatric patients

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Introduction: Metoclopramide (MCP) is a dopamine-receptor antagonist to stimulate gastric contractions for the treatment of gastroesophageal reflux disease (GERD). It is predominantly metabolized in liver by CYP2D6. For Asians, they usually have *CYP2D6*10* which leads to low affinity and expression of CYP2D6. The CYP2D6 genetic polymorphism has considerable effects on the pharmacokinetics of metoclopramide.

Aims. Based on data of our clinical trials, we developed a physiologically based pharmacokinetic (PBPK) model for *CYP2D6*wt/*wt* group and scaled to other genotypes and pediatric groups.

Methods: A 20 mg single oral dose of metoclopramide was given to forty-three healthy Korean subjects composed of *CYP2D6*wt/*wt (*wt = *1 or *2), CYP2D6*wt/*10, CYP2D6*10/*10 and CYP2D6*5/*10* genotypes. Blood samples were collected before and after drug administration of metoclopramide. The plasma concentrations of metoclopramide were measured using an HPLC-MS/MS system. Based on our clinical trials and physico-chemical parameters, PBPK model of metoclopramide was created using PK-sim® software. After that, a PBPK model was fitted the 99.998% confidence interval criteria (Abduljalil K et al, 2014) and validated by comparing with other clinical trials.

Results: We found meaningful differences in AUC_{end}, AUC_{inf}, oral clearance (CL/F) and C_{max} in CYP2D6 genetic polymorphism (P<0.0001, respectively). The C_{max} of clinical PK data were 26.8±9.2 (n=12), 37.6±6.4 (n=8), 44.3±9.2 (n=15) and 45.7±6.9 (n=8) in *CYP2D6*wt/*wt, *wt/*10, *10/*10* and *5/*10*, respectively. According to the developed PBPK model of metoclopramide, the observed PK parameters of adult were all within acceptance criteria, whereas in those of pediatric excluding CL/F met the acceptance criteria.

Discussion: The developed PBPK model of metoclopramide can be useful to personalized therapies for special patient groups and predict the effects in different CYP2D6 genotypes. In order to establish the accurate pediatric PBPK modeling of metoclopramide in CYP2D6 genotypes, PK/PD data needs to be further studied.

Abduljalil K et al (2014) Drug Metab Dispos 42(9):1478-1484.

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Prediction of flurbiprofen pharmacokinetics from using in vitro metabolism data in relation to CYP2C9 genetic polymorphism.

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Introduction: This present study focuses on the prediction of Flurbiprofen metabolism. AUC, C_{max}, T_{max}. Clearance (CL/F) and half-life ($t_{1/2}$) are primary parameters in physiologically based pharmacokinetic (PBPK) modeling. Flurbiprofen, phenylalkanoic acid derivative family of non-steroidal anti-inflammation drugs (NSAIDs), is used to treat inflammation in patients with arthritis. The metabolism of flurbiprofen consist of two different pathways; the 4-hydroxlation to 4-Hydroxyflurbiprofen and glucuronized to flurbiprofen glucuronide. The major metabolic pathway of flurbiprofen metabolized to 4'-hydroxyflurbiprofen is mediated by cytochrome P450 2C9 (CYP2C9) enzyme. Therefore, CYP2C9 enzyme activity primarily influences on flurbiprofen concentrations and flurbiprofen anti-inflammation effects. So CYP2C9 enzyme isoform is considered as a clinical parameter of flurbiprofen.

Aims. In this study, we simulate the effect of the *CYP2C9*3* allele on the pharmacokinetics of flurbiprofen and compared with clinical studies in healthy subjects with following phenotypes; *CYP2C9 *1/*1* and *CYP2C9 *1/*3*.

Method. 16 healthy Koran subjects with *CYP2C9 *1/*1* and *CYP2C9 *1/*3* genotypes were recruited in the present pharmacokinetic study of flurbiprofen. All subjects received 40mg single oral dose of flurbiprofen with 240mL water. Blood samples were collected up before the drug administration and 0.5, 1, 1.15, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hr after administration of flurbiprofen. In this study, PK-Sim® (PK-Sim® 7.4, Bayer AG, Wuppertal, Germany) was used to predict pharmacokinetics of CYP2C9 enzyme isoform. To verify the simulation, PK parameters were compared to monographic in vivo data and our flurbiprofen clinic research data. For assessment of Flurbiprofen PBPK model, an acceptable criterion based on observed human PK data is calculated using recently published Methods:

Results: In this study, AUC_{inf}, C_{max}, T_{max}, half-life ($T_{1/2}$), and clearance (CL/F) were calculated in modeling and met the model acceptance criterion (99.998% confidence interval). AUC_{inf} is significantly increased in *CYP2C9 *1/*3* model compared *CYP2C9*1/*1* model.

Discussion: Our flurbiprofen PBPK modeling requires further development, but it can contribute to the determination of the optimal flurbiprofen dosage considering inter-individual genetic differences.

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Should we be investigating inter-individual differences in biliary excretion of 5-Fluorouracil (5-FU) as a risk factor for 5-FU toxicity?

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Introduction: Severe to life-threatening gastrointestinal toxicity can occur in 10-20% of patients who receive 5-fluorouracil (5-FU) treatment. Metabolism by dihydropyrimidine dehydrogenase (DPD) eliminates >80% of a dose of 5-FU and up to 15% is renally eliminated as unchanged 5-FU. Thus impairment in either of these pathways can result in increased exposure to 5-FU and lead to severe 5-FU toxicity.

Aims. To explore whether differences in 5-FU exposure and toxicity in three patients could be explained by DPD status and/or renal function.

Methods: Patients (n=3) received 5-FU (370 mg/m^2 IV bolus). Blood samples were taken at 0, 0.5, 0.75, 1 and 1.5 hours post-dose. Plasma concentrations of 5-FU and metabolites were measured by LC-MS/MS. A cumulative (0-3h) urine sample was collected to determine fraction of dose excreted as unchanged 5-FU. Renal function was estimated from blood creatinine levels. Results from DPD genotype and phenotypic tests (thymine challenge & endogenous uracil) were assessed.

Results: One patient experienced severe 5-FU related gastrointestinal toxicity. This patient had >2-fold higher 5-FU exposure than the other patients (15.11 vs 6.33 & 7.03 mg.h/L). This patient did not display any signs of DPD metabolic deficiency with almost superimposable 5-FU metabolic profiles as the other patients. This also suggests no additional downstream metabolic deficiencies. This patient had renal function (CrCL) similar to the other patients and excreted similar amounts of unchanged drug in the urine ($Ae_{0\text{-}3h}$ 72.72 vs 58.32 & 77.12 mg).

Discussion: Elevated exposure to 5-FU in this patient could not be explained by deficient DPD activity or renal impairment. It may be possible that this patient had altered biliary excretion since previous studies have shown 5-FU can be eliminated in bile. However, inter-individual differences in 5-FU biliary excretion and the significance of this pathway for inter-individual differences in 5-FU exposure and risk of toxicity is currently unknown.

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The effects of CYP2C19 genetic polymorphism on the pharmacokinetics of omeprazole using physiology and physicochemistry based pharmacokinetic predictions.

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Introduction: Omeprazole, a proton pump inhibitor, is widely used to treat gastric acid-related disorders such as gastroesophageal reflux disease (GERD), peptic ulcer disease, and other diseases characterized by the over-secretion of gastric acid. It suppresses gastric acid secretion by specific irreversible inhibition of the H+/K+ ATPase enzyme system at the secretory surface of the gastric parietal cell. After absorption, omeprazole is primarily metabolized to inactive compound 5-Hydroxyomeprazole, 5'-O-Desmethyl omeprazole, Omeprazole-sulfone, and 3-Hydroxyomeprazole by cytochrome P450 (CYP) enzyme mediated pathway. We investigated the CYP2C19, the major metabolite enzyme of omeprazole, genetic polymorphism effects on drug metabolism using physiologically based pharmacokinetic (PBPK) modeling and compared with clinical studies in healthy subjects with following phenotype *CYP2C19 *1/*1, CYP2C19 *1/*2* and *CYP2C9*1/*3*.

Method: Twenty-nine healthy subjects were selected and they were divided into three different groups according to CYP2C19 genotype *CYP2C19 *1/*1, CYP2C19 *1/*2* and *CYP2C9*1/*3.* The development of omeprazole PBPK model using pharmacokinetic data of twenty-nine healthy Korean subject about CYP2C19 genetic polymorphism. In this study, PK-Sim® (PK-Sim® 7.4, Bayer AG, Wuppertal, Germany) was used to predict pharmacokinetics of CYP2C19 enzyme isoform. To verify the simulation, PK parameters were compared to monographic in vivo data and our omeprazole clinic research data. For assessment of omeprazole PBPK model, acceptable criterion based on observed human PK data is calculated using recently published Methods:

Results: In this study, AUC_{inf}, C_{max}, T_{max}, half-life ($T_{1/2}$), and clearance (CL/F) were calculated in modeling and met the model acceptance criterion (99.998% confidence interval. AUCinf is significantly increased in *CYP2C19 *1/*2* and *CYP2C19*1/*3* models compared C*YP2C19*1/*1* model.

Discussion: In this study, we can observe significant different of omeprazole pharmacokinetic parameters in relation to CYP2C19 genetic polymorphism. So, we expected to these studies can contribute to the determination of the optimal omeprazole dosage considering inter-individual genetic differences.

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Physiology and physicochemistry based pharmacokinetic predictions: effect of CYP2C9 genetic polymorphism on candesartan pharmacokinetics.

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Introduction: Candesartan cilexetil, an oral nonpeptide angiotensin II receptor antagonist with high selectivity for the AT1 receptor, is widely used to treat hypertension and heart failure. It is a prodrug that is rapidly converted to its active metabolite candesartan during absorption from the gastrointestinal tract. After conversion into its active metabolite, candesartan, during gastrointestinal absorption, it is further metabolized to the inactive compound O-Deethylated candesartan by CYP2C9-mediated pathway. We investigated the CYP2C9 enzyme genetic polymorphism effects on drug metabolism using physiologically based pharmacokinetic (PBPK) modeling and compared with clinical studies in healthy subjects with the following phenotype *CYP2C9*1/*1* and *CYP2C9*1/*3.*

Methods: Twenty-two healthy subjects were selected and they were divided into two different groups according to CYP2C9 genotype, *CYP2C9*1/*1* and *CYP2C9*1/*3*. After overnight fasting, each subject received a 16mg oral dose of candesartan cilexetil. The development of tramadol PBPK model using pharmacokinetic data of twenty-two healthy Korean subject about CYP2C9 genetic polymorphism. In this study, PK-Sim® (PK-Sim® 7.4, Bayer AG, Wuppertal, Germany) was used to predict pharmacokinetics of CYP2C9 enzyme isoform. To verify the simulation, PK parameters were compared to monographic in vivo data and our candesartan clinic research data. For the assessment of candesartan PBPK model, an acceptable criterion based on observed human PK data is calculated using recently published Methods:

Results: In this study, AUC_{inf}, C_{max}, T_{max}, half-life ($T_{1/2}$), and clearance (CL/F) were calculated in modeling and met the model acceptance criterion (99.998% confidence interval. AUC_{inf} is significantly increased in *CYP2C9 *1/*3* and *CYP2C9*1/*13* model compared *CYP2C9*1/*1* model.

Discussion: In this study, we only predict adult male subject pharmacokinetic parameters. Further studies we planned to predict in diverse age group and applied to personal precision medicine.

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Prediction of propranolol pharmacokinetics changes according to the variation in genetic and non-genetic factors

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Introduction: Propranolol, used to treat high blood pressure and irregular heart rate, is metabolized by cytochrome P450 2D6 (CYP2D6) to 4-hydroxypropranolol. Physiologically based pharmacokinetic (PBPK) modeling is a mathematical modeling technique for predicting the absorption, distribution, metabolism and excretion (ADME) of synthetic or natural chemical substances in humans and other animal species.

Aims. We investigated whether the pharmacokinetics of propranolol was altered by the different CYP2D6 genotypes in Korean subjects. And we developed the PBPK model of propranolol related to CYP2D6 genetic polymorphism.

Methods: Twenty-five volunteers were grouped as CYP2D6*wt/*wt (**wt=1* or 2, n=7), *CYP2D6*1/*10* (n=3), *CYP2D6*1/*5* (n=4), and *CYP2D6*10/*10* (n=11) according to their genotypes. Propranolol hydrochloride 40 mg (Pranol®) was administered orally once to each subject in these four groups. The PBPK modeling of propranolol was developed and optimized using PK-sim® software. And, validation of PBPK model was conducted by comparing the predicted values with observed values from comparison the pharmacokinetic studies.

Results: In clinical data, AUC_{end}, AUC_{inf}, C_{max} and oral clearance of propranolol were significantly different in CYP2D6 genetic polymorphism ($P<0.005$, respectively). According to physico-chemical parameters and ADME of each genotype, PBPK model of propranolol was developed. For metabolism, input values of in vitro V_{max} in the presence of recombinant enzyme for *CYP2D6*1* and *CYP2D6*10* were 1.610 and 0.180 pmol/min/pmol, respectively. And, input values of K_m for *CYP2D6*1* and *CYP2D6*10* were 3.080 and 23.430 μM, respectively. The AUC_{inf} mean of simulated PBPK model were 146.65, 188.03, 266.94 and 350.36 ng·h/ml in *CYP2D6*wt/*wt, CYP2D6*1/*10, CYP2D6*1/*5* and *CYP2D6*10/*10*, respectively. The developed PBPK model of propranolol successfully described the pharmacokinetics of each CYP2D6 genotype group and its simulated values were within acceptance criterion (99.998% confidence interval).

Discussion: These results demonstrate the possibility of propranolol prescription considering the individual genetic differences through this mechanical approach. In addition, the PBPK modeling of propranolol in relation to CYP2D6 genotypes will be applicable to the treatment of various ethnic groups, ages, and patients.

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Prediction of metoprolol pharmacokinetics changes according to the variation in genetic and non-genetic factors

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Introduction: Metoprolol is a beta blocker used in the treatment of hypertension, angina, and heart failure. Metoprolol is primarily metabolized by the CYP2D6 enzyme, which catalyzes alpha-hydroxylation and O-demethylation. The genetic polymorphism of CYP2D6 leads to differences in the pharmacokinetics of CYP2D6 substrates. It has been reported that *CYP2D6*10* allele is responsible for the pharmacokinetic variability of metoprolol seen in Asian. Physiologically based pharmacokinetic (PBPK) modeling is a useful tool for predicting the PK profile of drugs that assess the impact of covariates such as demographics, race, and genetic polymorphism.

Aims. We investigated whether the pharmacokinetics of metoprolol was altered by the different CYP2D6 genotypes in Korean subjects. And we developed the PBPK model of metoprolol related to CYP2D6 genetic polymorphism.

Methods: Forty-five volunteers were recruited and grouped as *CYP2D6*wt/*wt* (**wt=1* or 2, n=15), *CYP2D6*wt /*5* (n=4), *CYP2D6*wt/*10* (n=9), *CYP2D6*10 /*10* (n=10), *CYP2D6*5/*10* (n=5) and *CYP2D6*5/*5* (n=2) according to their genotypes. Metoprolol tartrate 100 mg (Betaloc®) was administered orally once to each subject in these six groups. The PBPK modeling of metoprolol was developed and optimized using PK-sim® software. And, PBPK model validation was conducted by comparing the predicted values with observed values from comparison the pharmacokinetic studies.

Results: The AUC_{inf} mean of clinical PK data were 492.96, 673.57, 790.15, 2054.61, 3157.16 and 5152.65 ng·h/ml in *CYP2D6*wt/*wt, *wt/*10, *wt/*5, *10/*10, *5/*10* and *5/*5*, respectively. According to physico-chemical parameters and ADME of each genotype, PBPK model of metoprolol was developed using PK-sim® software. The developed PBPK model of metoprolol successfully described the pharmacokinetics of each CYP2D6 genotype group and its simulated values were within acceptance criterion (99.998% confidence interval).

Discussion: We developed a PBPK model of metoprolol with regard to genetic polymorphism, which predicts the pharmacokinetics of metoprolol, considering demographic data of subjects, physico-chemical parameters, ADME properties, and CYP2D6 genotypes. These results demonstrate the possibility of metoprolol prescription considering the individual genetic differences through this mechanical approach. Furthermore, the PBPK modeling of metoprolol in CYP2D6 genotypes will be applicable to the treatment of various races, ages, and patients.

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Attitudes of healthy volunteers to genetic testing in phase I clinical trials

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Introduction: Genetic testing is an optional part of many clinical trials. This introduces several issues for potential participants to consider prior to consent, including genetic security, genetic privacy, appropriateness of genetic re-analysis, and the personal consequences of incidental genetic findings.

Aims. To investigate the attitudes of healthy volunteers to genetic testing in phase I clinical trials.

Methods: Anonymous paper surveys were distributed to healthy volunteers in the waiting room of a clinical trial facility. There were 9 questions about the importance of genetic security, genetic privacy, genetic re-analysis and incidental genetic findings. Questions were written using 5-point Likert scales, visual analogue scales, polar responses (YES or NO), and rankings (1-5).

Results: A total of 275 participants (86.8% participation rate) completed the survey from May to September 2019. The mean age was 27 years, 54% were male, 62% had not previously done a clinical trial, and 55% indicated completing education to the level of high school or above. Participants generally favoured the return of incidental genetic findings (60.0% selected 'important' or 'very important'). However, there was no clear threshold of the disease risk required for this to occur. Genetic security and genetic privacy were 'important' or 'very important' issues for 55.5% and 56.9% of participants, respectively. Most participants wish to know about future genetic re-analyses (89.4%). There were no statistically significant relationships between demographic data and the attitudes to genetic testing.

Conclusions. Most healthy volunteers are happy to undertake genetic testing as part of their participation in phase I clinical trials. However, they wish to know about future genetic testing on stored samples. About half of phase I volunteers identify issues around genetic testing as being important, whilst the other half are indifferent.

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Pharmacogenetic variants and psychological factors do not explain self-reported antidepressant side effects.

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Introduction: Many patients prescribed an antidepressant stop taking it because of side effects. Genetic factors involving drug metabolism, and psychological factors including state or trait anxiety, may explain variation in side effect outcomes.

Aims. Our aim was to examine the relative contribution of genetic and psychological factors in people with self-reported antidepressant side effects.

Methods: We undertook a case control study (n=194) of people who took a selective serotonin reuptake inhibitor (SSRI) or serotonin / noradrenaline reuptake inhibitor (SNRI) in the past 2 years, recruited via social media advertising. Cases had previously not tolerated at least one trial of an SSRI or SNRI, evidenced by stopping the drug or reducing the dose by at least 50% because of a side effect. Control participants had taken an SSRI or SNRI but did not meet case criteria. Variation in the genes *CYP2D6, CYP2C19* and *CYP2C9* was analysed by Sanger sequencing on DNA extracted from blood or saliva. Participants also completed the Short Health Anxiety Inventory-18, Kessler Psychological Distress Scale (K10) and NEO-FFI-3 personality questionnaire.

Results: Participants were predominantly (87.1%) female. 70.8% had a current K10 score of 22 or more, indicating current high psychological distress. There was no difference in CYP2D6, CYP2C19 or CYP2C9 phenotype distribution between cases and controls. Cases were younger than controls (d=0.41, p=.005) but there was no consistent evidence they had higher psychological distress, health anxiety or neuroticism.

Discussion: There was low correspondence between participants' CYP2D6, CYP2C19 and CYP2C9 phenotypes and their history of antidepressant tolerability. A history of not tolerating SSRI or SNRI therapy was not associated with variation in the pharmacogenes we tested, nor was it associated with health anxiety or neuroticism.

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The optimization of voriconazole dosing related to CYP2C19 major alleles in healthy Asian.

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Introduction: Voriconazole acts as an enzyme inhibitor blocking the synthesis of ergosterol and thereby the growth of the microorganism. And since it is used mostly in patients with severe fungal infections with high mortality, appropriate administration is required depending on the concentration of the patient's blood medication. It is mainly metabolized via the hepatic cytochrome P450 (CYP) isoenzyme 2C19 (CYP2C19) and to a lesser extent by CYP3A4 and CYP2C9. So, preferentially We figured out the CYP2C19 enzyme genetic polymorphism effects on drug metabolism using physiologically based pharmacokinetic (PBPK) modeling and compared with clinical studies in healthy subjects with following phenotype *CYP2C19*1/*1, CYP2C19*2/*2* in between Chinese and Koreans.

Aims. The purpose of the study is to identify differences of CYP genotyping in both Korean and Chinese and to present individually precision medicine with the corresponding individual plasma concentration of drug.

Methods: According to the investigation, twelve subjects were selected from South Koreans and fourteen from China, respectively. And they were divided into two different groups according to CYP2C19 genotype, *CYP2C19*1/*1, CYP2C19*2/*2.* Each subject received a 200 mg oral dose of voriconazole. And then, venous blood samples were collected and quantified by LC-MS/MS. In this study, comparison between predicted value and observed value was evaluated by two-fold error which is widely used for acceptable prediction in PBPK model evaluation. Finally, PK-Sim® was used to predict pharmacokinetics of CYP2C19 enzyme isoform.

Results: In this study, AUC from dosing to the time of the end (AUC_{end}), AUC from dosing to time infinity (AUC_{inf}), the maximum observed plasma concentration (C_{max}), the time to achive Cmax (T_{max}), $t_{1/2}$, and CL were calculated in modeling and met the two-fold error. As a result, a person with a genotype of CYP2C19*2/*2 is recommended to consume approximately 56% less voriconazole than a person with a genotype of CYP2C19*1/*1, according to the dose optimization formula[$(AUCend^{*1*/1}/AUC^{end*2/*2}-1) \times 100$], because the AUC_{end} was approximately twice as different.

Discussion: Since validation was done for healthy people, we hope that precision medicine will be applied to all ages by predicting values in children and elderly people based on the disposition of the voriconazole that was considered in this study.

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A QPCR-block assay for the detection of inter-individual differences in melphalan-induced DNA damage.

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Introduction: Melphalan myeloablation, followed by autologous stem cell transplantation, is used to treat multiple myeloma. At standard dosages, inter-individual differences in response and mucosal toxicity occur. Melphalan elicits its cytotoxic effects by covalently alkylating DNA, inhibiting replication/transcription. Assessment of *ex vivo* melphalan adducts in peripheral blood mononuclear cells (PBMC) using a long-range QPCR-block assay has been reported to associate with melphalan response1. Our previous work demonstrated that this long range polymerase assay results in non-specific amplification of the target sequence, lacks precision and was incompatible with PBMC cell lysate.

Aims. To improve the previously published assay for detection of DNA adducts in PBMC exposed to melphalan *ex vivo* and undertake a preliminary assessment of inter-individual differences in adduct formation.

Methods: Following informed consent (16/NTA/239), peripheral blood samples were drawn from volunteers. Either purified genomic DNA or PBMC were exposed to a range of melphalan concentrations for 1 h at 37°C. Aliquots of DNA or cell lysate were used as template for PCR amplification of a 1.6 kb TP53 region using *Taq* polymerase. Quantification of the amplicon was by gel densitometry or fluorescence spectrophotometry and the number of adducts/kb were calculated relative to untreated control

Results: Both methods could detect changes in PCR amplification. However, fluorescence spectrophotometry was quantitative, had improved precision (CV 12% versus 39%) and higher throughput (75 samples/plate versus 18 samples/gel). Using this improved method, reproducible inter-individual differences in the number of adducts (mean ± SD) formed in melphalan-exposed PBMC could be detected across n= 9 donors (range: 3.13 ± 0.014 to 5.84 ± 0.021, adducts.kb-1ng.ml-1).

Discussion: This assay increases the feasibility for studying inter-individual differences in formation and/or rate of repair of melphalan adducts in a clinical setting using an easily accessible surrogate tissue.

1. Stefanou DT et al (2012) Br J Clin Pharmacol 74(5):842-53.

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PNG HIV/AIDS patients have unique genetics

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Introduction: Papua New Guinea (PNG) has the highest prevalence of HIV/AIDS in the Pacific with efavirenz (EFV) as the main treatment. Genetic variability in *CYP2B6* (EFV metabolism) and *NR1I2* & *NR1I3* (regulators of CYP expression) has been associated with altered plasma EFV concentrations and CNS/Psychiatric toxicities in Caucasian and African populations, but little is known about these genes in PNG. We previously showed unique CYP2B6 genetics in this population. We hypothesised that additional polymorphisms in *CYP2B6, NR1I2* and *NR1I3* will (1) have a different frequency in PNG than in other populations and (2) be associated with efavirenz CNS or Psychiatric adverse effects in PNG HIV/AIDS patients.

Aims. To determine the frequencies of *CYP2B6* (516C>T, 750T>C, 983T>C, 15582C>T), *NR1I2* (8055C>T, 63396C>T, 25385C>T) and NR1I3 (540C>T, 8784T>C) SNPs in PNG HIV/AIDS patients receiving EFV; to compare the data to other populations, and to examine genotype differences in the incidence of CNS or Psychiatric adverse effects.

Methods: Demographic and clinical data, including CNS and Psychiatric adverse effects, and saliva were collected from 51 PNG HIV/AIDS patients. Salivary DNA was genotyped by Agena MassARRAY and allele frequencies compared to other populations from the 1000 Genomes Project (Auton et al., 2015) by Fisher's exact test. Incidence of CNS or Psychiatric adverse effects was compared between genotypes by Chi-squared or Fisher's exact tests.

Results: In addition to CYP2B6 516G>T, PNG HIV/AIDS patients have a significantly higher *NR1I3* 8784T>C (76%) variant allele frequency compared to other populations (44-57%, $p<0.05$). There were no significant genotype differences in CNS or Psychiatric adverse effects ($p>0.71$).

Discussion: PNG HIV/AIDS patients exhibit a unique pattern of genetic variability in key EFV metabolism genes. No significant association with EFV adverse effects was detected in this small study, and larger studies incorporating efavirenz PK are required.

Auton et al. (2015) Nature 526:68-74.chi