A1

Synthesis of polymeric iNOS antagonist prodrug for targeted inhibition of inflammation induced angiogenesis.

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A2

From a phase III clinical vascular disrupting agent to a target based immuno-modulator, the rise of xanthenones

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Introduction. Tumour-associated-macrophages have immuno-suppressive and pro-tumourigenic functions, identifying them as an immunomodulatory drug target in cancer therapy. Macrophages and monocytes depend on signalling through the cell surface receptor tyrosine kinase colony stimulating factor 1 receptor (CSF1R) for survival and proliferation. Blocking CSF1R cell signalling activity can deplete macrophage populations and generate an anti-tumour effect in pre-clinical models. We discovered that the unsuccessful phase III clinical agent Vadimezan had the ability to act as broad-spectrum kinase inhibitor alongside its known vascular disrupting effects and its recently established effect as a murine-immuno-stimulator.

Aim. To develop Vadimezan into a novel kinase inhibitor with immuno-modulatory activity.

Results. Inspired by the 3-dimensional structure of CSF1R kinase domain, we used a combination of protein-structure guided drug design, medicinal chemistry and molecular pharmacology to transform this lead molecule into a potent, highly selective CNS sparing CSF1R blocker.

Discussion. Our new CSF1R inhibitor represents a new type of kinase inhibitor suitable for macrophage modulation in *in vivo* tumour biology.

A3

Using Bayesian software to inform initial busulfan dose in adults receiving haematopoietic stem cell transplantation

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Introduction. Therapeutic drug monitoring of busulfan is recommended to both optimise dosing and minimise the risks of graft rejection and toxicity such as sinusoidal obstruction syndrome. Busulfan conditioning for haematopoietic stem cell transplantation typically comprises four once daily weight-based intravenous doses. Using Bayesian software to guide the initial busulfan dose may result in more patients achieving the target AUC_{24}. It is often necessary to repeat busulfan concentration monitoring in those who fail to meet the target, therefore adopting a model-based approach to initial dosing may lead to reductions in the number of patient interventions, with ensuing savings in costs, resources, and clinician time.

Aims. To assess whether using Bayesian software (NextDose) to inform the initial busulfan dose for adults undergoing haematopoietic stem cell transplantation improves the proportion of patients meeting the target AUC_{24}, compared to the standard weight-based dosing approach.

Methods. Retrospective data (including measured busulfan concentrations) were compiled from adults who had haematopoietic stem cell transplantation between 2011 and 2022 with busulfan conditioning using once daily intravenous dosing (3.2 mg/kg for four days). The data from these patients were used to simulate the busulfan concentrations resulting from an initial dose informed by NextDose. The proportion of patients reaching the initial dose target (AUC_{24} of 22.5 mg/L.h +/- 20%) with each approach was calculated and compared using McNemar's test.

Results. Seventy-seven patients were eligible for inclusion. Their median (range) age was 43 years (17-61 years) with weight 75 kg (52-130 kg); 60% were males (46/77). The median (range) weight-based initial dose was 3.2 mg/kg (2.2-3.3 mg/kg). With weight-based initial dosing, 36% (28/77) of patients achieved the target AUC_{24}. The simulated concentrations from model-based initial dosing showed that 51% (39/77) would have met the target AUC_{24} (McNemar's $X^2(1) = 6.7$, $p = 0.007$). Of those patients outside the AUC_{24} range, the majority were under-target regardless of dosing method: 96% with weight-based dosing and 95% with model-based dosing.

Discussion. Using Bayesian software to inform the initial dose significantly improved the proportion of patients attaining the target first dose AUC_{24}, potentially leading to resource savings.

A4

An Old Drug with New Tricks; Examining the Action of Metformin in ALK+ Lung Cancer.

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Introduction. Lung cancer has the greatest incidence of cancer mortality, with approximately 1.7 million deaths/year. The largest proportion of lung cancer cases are classified as non-small cell lung cancer (NSCLC). Receptor tyrosine kinases are frequently oncogenic drivers in NSCLC, which includes the ALK receptor (occurring in ~5-7% of NSCLC cases). ALK inhibitors, such as crizotinib, are potent agents of cell death in ALK NSCLC, however, resistance typically occurs around 12 months after commencing treatment. To overcome this resistance, novel strategies are being explored, which includes repurposing existing drugs. The hypoglycaemic agent, metformin, has been epidemiologically shown to reduce the risk of cancer. Although the effect has been explored in other cancers, there is limited data on the value of metformin in ALK+ NSCLC.

Aims. This study aimed to examine if metformin, crizotinib and a combination of crizotinib and metformin in cellular and animal models of ALK+ NSCLC.

Methods. ALK+ H3122 cells were used to determine the cell viability after drug treatment using the sulforhodamine B assay. Western blotting was used to examine ALK, mTOR and AMPK and confocal microscopy using the mitotracker red dye were utilised to examine mechanisms of metformin in H3122 cells. To examine metformin *in vivo*, tumour-bearing Nu/J mice were orally administered either the vehicle control, crizotinib (25 mg/kg), metformin (100 mg/kg) or the combination every day for 2 weeks. Tumour volume was measured daily.

Results. *In vitro* metformin only enhanced the cytotoxicity of low concentrations of crizotinib and provided no additional effect at higher concentrations. Crizotinib inhibited the phosphorylation of ALK, but metformin did not, confirming the two drugs act on different targets. Metformin did not appear to act on mTOR nor AMPK, however, did reduce mitotracker staining (suggesting a reduction in the mitochondrial membrane potential), indicating that the likely mechanism is via the mitochondria. *In vivo* metformin, crizotinib and the combination all reduced tumour growth, however, the combination was no greater than crizotinib alone.

Discussion. Metformin was efficacious in ALK+ NSCLC models, however, did not provide added benefit to crizotinib. Nevertheless, these findings warrant further investigation into the value of metformin in lung cancer therapy.

A5

Omeprazole treatment failure in gastro-oesophageal reflux disease and genetic variation at the *CYP2C* locus.

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Introduction. Omeprazole is extensively used to manage gastro-oesophageal reflux disease (GORD). It is primarily metabolized by CYP2C19. The *CYP2C19*17* (rs12248560) allele and the recently described *CYP2C:TG* haplotype (rs11188059 and rs2860840) are associated with increased enzymatic activity, and may reduce omeprazole exposure.
Aims. This observational study aims to investigate the association between *CYP2C19*17* allele and *CYP2C:TG* haplotype with omeprazole treatment failure in GORD.
Methods. We recruited GORD patients who either did not respond to, or experienced breakthrough reflux symptoms, with omeprazole 40-80 mg/day for a minimum of 8 weeks. The GerdQ score was used to gauge symptomatic severity (range 0-18, higher means greater severity). DNA was extracted from blood or saliva samples. Sanger sequencing were performed for genotyping and haplotyping. Fisher's exact tests were used to compare proportions.
Results. A total of 55 cases (94.5% NZ European ethnicity) were recruited with a median age (range) of 56 years (19-82) and GerdQ score of 11 (5-17). Of these, 21 (38.2%) had at least one **17* allele with 19 (34.5%) heterozygotes and two (3.6%) homozygotes. Our cohort contained 30 (27.3%) *CYP2C:TG* haplotypes with seven (12.7%) *TG/TG* carriers, and 16 (29%) carrying one *TG* haplotype. Compared with known European population allele and haplotype frequencies, no significant differences were observed for *CYP2C19*17* allele (21%), *CYP2C19*17* homozygotes (ultrarapid metabolisers) (3.6%), *CYP2C:TG* haplotypes (27.3%), and *CYP2C:TG* heterozygotes (29%) ($p > 0.05$ for all comparisons). Gastroscopy and 24-hour oesophageal pH/impedance tests demonstrated objective evidence of GORD in a subgroup of 39 (70.9%) cases, in which the *CYP2C:TG/TG* was significantly enriched ($p=0.03$), but not the *CYP2C19*17/*17* ($p>0.99$).
Discussion. Our results suggest that omeprazole treatment failure in GORD is associated with *CYP2C:TG/TG*, but not *CYP2C19*17*.

A6

The dual benefits of $5HT_3$ receptor antagonists in cancer therapy

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A7

Examining the Effect of the Novel Cardioprotective Agent oCOm-21 on the NLRP3 Inflammasome in an *Ex Vivo* Rat Model of Cardiac Ischaemic Reperfusion Injury.

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Introduction. Life-saving coronary artery bypass and vascular grafting (CABG) procedures are associated with a deleterious ischaemic reperfusion injury (IRI), which promotes progressive cardiac hypertrophy, fibrosis, and cardiomyocyte cell death. Prophylactic pharmacological ischaemic preconditioning agents show promise in reducing IRI severity. One proposed agent is a carbon monoxide releasing molecule oCOm-21, which has previously been shown to provide cardioprotection *ex vivo* in normotrophic and hypertrophic CYP1A1-Ren2 rat hearts. The mechanism of oCOm-21 remains elusive, with literature suggesting inhibition of the NLRP3 inflammasome may be involved. NLRP3 is activated through sterile inflammatory signals, facilitating the association of NLRP3 with the ASC adaptor protein. ASC facilitates caspase-1 activation, allowing the cleavage and secretion of the pro-inflammatory cytokines IL-1β and IL-18, triggering inflammatory cell recruitment, and subsequent myocardial fibrotic scarring.

Aims. To examine the effect of oCOm-21 on the expression of NLRP3, ASC and IL-1β in normotrophic and hypertrophic rat hearts.

Methods. NLRP3 and ASC were examined using immunofluorescence in oCOm-21 (1 and 3 μM) treated normotrophic and hypertrophic CYP1A1-Ren2 rat hearts (n=5). Western blotting was used to examine the expression of active IL-1β and pro-IL-1β (n=2).

Results. oCOm-21 (3 μM) resulted in 36.4% and 31.0% reductions in the NLRP3 intensity in normotrophic and hypertrophic hearts, respectively. ASC intensity remained consistent between all groups. Pearson's correlation analysis suggested that oCOm-21 (3 μM) significantly reduced the co-localisation of NLRP3 and ASC in hypertrophic cardiac tissue by 46.3%. Preliminary results from western blot analysis suggests the ratio of active IL-1β to pro-IL-1β is reduced in normotrophic and hypertrophic hearts treated with oCOm-21 (3 μM).

Discussion. Current findings suggest that 3 μM oCOm-21 delivered prophylactically immediately prior to IRI in an isolated rat heart may reduce the formation of NLRP3 protein and the assembly of the NLRP3 inflammasome, and subsequently inhibit the catalytic activity of NLRP3.

A8

Delineating the interactions between the cannabinoid CB_2 receptor and its regulatory effectors; β-arrestin 2 and G protein-coupled receptor kinases.

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Introduction. The cannabinoid CB_2 receptor (CB_2) is a promising therapeutic target for immune and inflammatory conditions. Classically, G protein-coupled receptors (GPCRs), like CB_2, are regulated by G protein-coupled receptor kinases (GRKs), enzymes which phosphorylate intracellular residues of the receptor, and subsequently modulate β-arrestin recruitment. However, little is currently known of the interactions and processes that underpin CB_2 desensitisation and regulation by these effectors.

Aims. This study characterised the role of six GRK isoforms in β-arrestin 2 recruitment to CB_2. Mutagenesis of several distal CB_2 C-terminal aspartic acid residues was also performed to assess additional structural elements that may be involved in the regulation of CB_2.

Methods. Real-time BRET biosensor assays were used to measure β-arrestin 2 translocation and G protein dissociation in CB_2-expressing HEK cells.

Results. Surprisingly, overexpression of the GRK isoforms 1-6 did not considerably improve β-arrestin 2 translocation to CB_2. Consistent with this, inhibition of endogenous GRK2/3 did not substantially reduce β-arrestin 2 translocation. This led us to speculate that aspartic acid residues in the C-terminus of CB_2 may act as phospho-mimetic residues, enabling the recruitment of β-arrestins in the absence of GRK phosphorylation. Mutagenesis of C-terminal aspartic acid residues attenuated β-arrestin 2 translocation, with small effects on G protein desensitisation.

Discussion. These findings suggest CB_2 may not follow the classical mechanism of GPCR GRK-mediated desensitisation. C-terminal aspartic acid residues may act as surrogates for phosphate groups to enable β-arrestin activation, although the functional significance of these phospho-mimetic residues remains unclear. The insights gained into the regulatory mechanisms of CB_2 from this study may assist our understanding of drug tolerance and dependence.

A9

Investigating G protein vs β-arrestin bias of late-generation synthetic cannabinoids at the CB1 receptor

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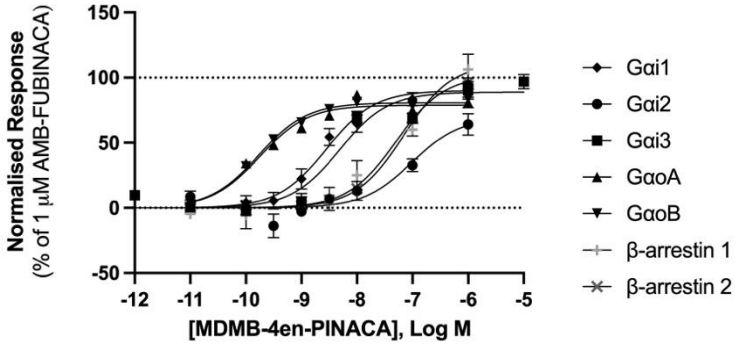

Introduction. The type 1 cannabinoid receptor (CB1) is a Gi/o-coupled receptor responsible for producing the effects caused by drugs such as Δ^9-tetrehydrocannabinol (THC) and the infamous synthetic cannabinoid receptor agonists (SCRAs). SCRAs have been implicated in many instances of death and toxicity worldwide. This has led to the hypothesis that biased agonism may have role in the different levels of toxicity seen between SCRAs and THC.

Aims. To characterise the G protein subtype selectivity and β-arrestin recruitment for late-generation SCRAs and analyse the data for bias using the operational model.

Methods. Two BRET-based assay paradigms were used to quantify the G protein dissociation (Gαi1, Gαi2, Gαi3, GαoA, GαoB) and β-arrestin translocation caused by a panel of structurally distinct SCRAs alongside THC.

Results. Each compound was found to have a unique signalling profile, however statistical analysis revealed THC to prefer Gαi1 over Gαi3; 4CN-MPP-BUT7IACA showed a preference for β-arrestin 1 and 2 over GαoA and GαoB. Finally, 4F-MDMB-BUTINACA showed a preference for β-arrestin 2 over Gαi2.

Discussion. These results extend previous findings by our group, and others, to reveal the differences in signalling between THC and SCRAs. The quantification of the more proximal G protein dissociation in place of cAMP inhibition avoids signal amplification and grants insight into the G protein subtype selectivity of each compound.

A10

The predictive performance of an allopurinol adherence monitoring tool for clinical trials

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Introduction. Adherence to allopurinol in gout patients participating in clinical trials is often suboptimal increasing the risk of biased results and data interpretation challenges. A tool to identify poor adherence has been developed based on oxypurinol plasma concentrations, allopurinol's active metabolite [1]. The tool includes a series of oxypurinol concentration thresholds, below which poor adherence is assumed, and adjusted for daily dose, creatinine clearance, diuretic use and ethnicity. The predictive performance of the tool against externally collected data is unknown.

Aims. To externally evaluate an allopurinol adherence tool based on steady-state oxypurinol plasma concentrations.

Methods. The predictive performance of the adherence tool was assessed using data from the CKDfix study [2]. Data from n=184 subjects including paired oxypurinol plasma concentrations and pill counts, sampling times, age, serum creatinine, weight, height, diuretic status, eGFR, and daily dose were available. Subjects were considered adherent to therapy if the 'percent of tablets used' between clinical visits was ≥80%. For the purposes of this analysis, this value defined the 'true' adherence status of the subject. If the paired oxypurinol concentration at the same clinic visit was greater than the adherence threshold defined by the tool, it was assumed that the tool had identified an adherent subject, while concentrations below the threshold were assumed to detect suboptimal adherence. Predictive performance was assessed using sensitivity, specificity, negative and positive predictive values (PPV, NPV), and receiver operating characteristic (ROC) area under the curve (AUC).

Results. The allopurinol adherence tool produced sensitivity and specificity values of 76% and 87% respectively and a ROC AUC of 0.811. PPV and NPV were found to be 76% and 86% respectively.

Discussion. A tool to identify gout patients in clinical trials with suboptimal adherence using plasma oxypurinol concentrations was evaluated against externally collected clinical trial data. The predictive performance of the tool was found to be suitable for adherence screening in clinical trials.

[1] Smith-Diaz et al (2022) Brit J Clin Pharmacol. In press.
[2] Badve SV et al (2020) N Engl J Med 2020;382(26):2504-13.

A11

Novel thermoresponsive hydrogels (NTH) to prevent reactive gliosis after stroke

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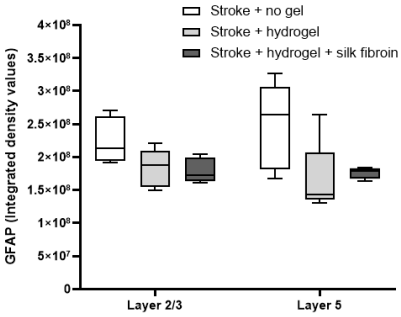

Introduction. Stroke is the leading cause of adult disability worldwide. The primary reason for the lasting functional impairment following stroke is the brain's limited ability to regenerate after an injury. Treatment measures such as systemic delivery of drugs and growth factors is limited by blood brain barrier (BBB) and off-target effects. Recently, localized delivery of therapeutic molecules by biopolymer hydrogels have gained significant attention for the treatment of stroke.

Aims. In the present study, silk fibroin was combined with chitosan to prepare a novel injectable thermoresponsive hydrogel (NTH).

Methods. The NTH was physically, mechanically, and chemically characterised to evaluate the suitability for injection into the brain. MTT assay and live-dead assay were performed to assess *in vitro* cytocompatibility using PC12 cells. Finally, NTH was injected into the infarct cavity of an ischemic stroke model to investigate *in vivo* biocompatibility and effects on reactive astrogliosis.

Results. Rheological analysis demonstrated that NTH retains a similar mechanics to that of brain tissue (~300 Pa) with an osmolality equivalent to that of cerebrospinal fluid (~290 mmol/kg). The *in vitro* cytocompatibility testing showed that NTH is nontoxic to PC12 cells with ~99% cell viability. The *in vivo* assessment revealed a smaller infarct cavity and decreased reactive astrogliosis as shown by the expression of glial fibrillary acidic protein (GFAP) and Iba1 in the peri-infarct region.

Discussion. These results suggest that NTH can be combined with drugs or growth factors as a potential therapeutic approach for stroke recovery.

A12

Differential interaction of β-arrestin for two CGRP-responsive receptors

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A13

Genomic analysis of angiotensin converting enzyme inhibitor - induced angioedema.

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Introduction: Angioedema is a rare but serious adverse drug reaction associated with angiotensin converting enzyme inhibitors (ACEi). Prior research has indicated that specific ethnic populations are at a higher risk of ACEi – induced angioedema (ACEi–A), and several hereditary forms of angioedema exist, suggesting a genetic link. Several candidate gene and genome wide association studies have been conducted for ACEi-A, but no candidate genes or genetic variants of major effect have been identified.

Aims: Using a model of monogenic predisposition to this adverse reaction, we set out to find variants that were rare in the population but highly enriched in ACEi-A.

Methods: To achieve this, we conducted whole exome sequencing (WES) or whole genome sequencing (WGS) on 20 ACEi-A cases.

Results: We identified one synonymous exonic variant in the *ACE* gene (rs4365) that was present in 40% of our ACEi-A cases compared to 4% of our controls (p-value=1.7e-05) and 3% of the gnomAD total dataset (p-value <2.2e-16). This SNP is located within or close to an enhancer element that appears to regulate ACE and other genes. Using the WGS data, we also sought variants in candidate genes previously implicated in drug induced or hereditary angioedema, but found no evidence for consistent involvement of such variants in our cohort. In a separate analysis on the same dataset, we applied SKAT-O to evaluate rare variants within a larger set of genes linked to angioedema, and the main finding here was that aggregated variants in *ACE2* were nominally associated with reduced risk of angioedema. Finally, we examined copy number variants (CNV) called from the WGS data, and identified several rare duplication/deletion events in ACEi-A cases. A duplication encompassing the gene *ADGRE1* was present in three angioedema cases, although this CNV is rare in the wider population.

Discussion: this analysis identified several interesting potential genetic contributors to ACEi-A, however, the small cohort size limits statistical power and these findings need to be evaluated in larger cohorts, work which is in progress.

A14

Testing for dihydropyrimidine dehydrogenase deficiency in New Zealand to improve the safe use of 5-fluorouracil and capecitabine in cancer patients

Nuala Helsby
University of Auckland

Inherited dihydropyrimidine dehydrogenase deficiency is a rare disorder and approximately 3% of people of European ancestry have a partial deficiency in this enzyme. These individuals are at considerably increased risk of life-threatening adverse events when dosed with 5-FU or capecitabine (which forms 5-FU) for treatment of gastrointestinal or breast cancer. There are four well established risk variants within the DPYD gene which encodes this enzyme. Consensus guidelines for genotype-guided dosing of 5-FU and capecitabine have existed for a number of years, and despite evidence of clinical and cost effectiveness the implementation of this type of personalised medicine has not been widely adopted. My talk will cover the current state of knowledge about both genotype and phenotype testing as well as the recent recommendations by agencies and professional societies. I will also highlight some of the other factors which influence the safe use of this drug.

A15

Cannabinoids as Opioid Sparing Agents

Bridin Murnion
University of New Castle

The prescription opioid epidemic has resulted in dramatic increases in the adverse events of opioid dependence and death from opioid toxicity in the USA, Canada and Australia. Various regulatory and pharmacological interventions have been adopted in response to this. Use of prescribed and non-prescribed cannabis is widely promoted as opioid sparing, and as such is proposed to reduce opioid related adverse events. The pre-clinical and clinical data present a complex picture, to which the diverse pharmacology of cannabinoids contributes. These data will be reviewed and the research, clinical and regulatory challenges will be explored.

A16

The effect of mandatory prescription indications for antibacterials

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Introduction. The indication for a medicine is part of shared information between health professionals regarding treatment. Indications are poorly recorded in prescriptions at Canterbury District Health Board (CDHB) hospitals. Indications are 'optional' on paper prescriptions but can be mandated in electronic prescriptions. However, mandatory fields in electronic systems are often populated by inappropriate text.

Aims. To assess the effect of mandatory prescription indications on data quality.

Methods. Documented indications for all antibacterial prescriptions were extracted from the CDHB electronic prescribing and administration (ePA) system for four weeks before and four weeks after mandating indications for nine antibacterial medicines (the intervention group). Indications were manually classified as 'credible indication', 'other text', 'rubbish text', or 'blank'. The change in proportion of 'credible indications' for the intervention group after mandating indications was compared to a reference group of 94 antibacterials: 23 with and 71 without pre-existing mandatory indications.

Results. The proportion of prescriptions with 'credible indications' increased from 12.5% (270/2166) before to 77.3% (1684/2179) after mandating prescription indications in the intervention group. In comparison, 'credible indications' for antibacterial prescriptions without mandatory indications were 22.0% (793/3611) before and 46.0% (1667/3621) after, and antibacterial prescriptions with pre-existing mandatory indications were 80.3% (191/238) before and 83.2% (183/220) after, the intervention. For prescription indications in the intervention group, 'blank' decreased from 82.1% to 6.1%, 'rubbish text' increased from 0.05% to 1.3%, and 'other text' increased from 5.4% to 15.3%.

Discussion. Mandatory prescription indications in a hospital ePA system increased 'credible indications' for antibacterial prescriptions several fold. This was achieved in the context of an antimicrobial stewardship initiative. The small increase in 'rubbish text' and 'other text' illustrates the need for continuous monitoring and feedback to support good prescribing and communication.

A17

Evaluation of the appropriateness of the prior for incorporation into therapeutic drug monitoring software – an application to Infliximab

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Introduction. Dose individualisation of infliximab through therapeutic drug monitoring (TDM) is recommended in the NZ guidelines for IBD. Currently there are no standard TDM software that include infliximab. A critical step in developing a TDM approach is the identification and incorporation of an appropriate prior model.

Aim. To evaluate the appropriateness of prior studies of infliximab population pharmacokinetics for incorporation into a TDM application.

Methods. Real-world patient level concentration-time data were available from Christchurch (Ethics approval was obtained) – these patients were termed the target population. The literature was reviewed for population infliximab pharmacokinetic models. Each model was evaluated for appropriateness as a prior based on 4 criteria: (1) reproducibility of the published model, (2) exchangeability between the prior and the target populations, (3) the predictive performance of the prior into the target data and (4) clinical implications of applying the prior.

Results: Four population pharmacokinetic analyses were identified for infliximab. (1) only one study was able to be reproduced. (2) This study provided moderate evidence of exchangeability between the prior and target population. (3) The prior showed appropriate predictive performance when used to predict into the target population data. (4) If the prior had been used in a TDM application the decisions would have been the same as that found by a full population analysis of the target population.

Discussion: This is the first formal evaluation of the appropriateness of a prior for incorporation into a TDM application. In the example here, the prior was considered to be sufficiently similar to the target (fulfilling 3 of the 4 criteria) and therefore could be incorporated into the NextDose software for individualizing patients with inflammatory bowel disease in hospital.

A18

Exploring Go / No-Go with Oral Docetaxel plus Encequidar

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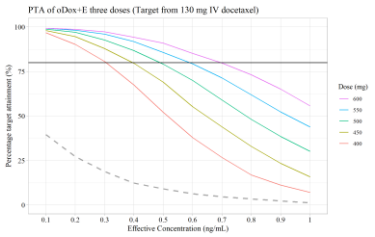

Introduction. Docetaxel is a synthetic taxane used in the treatment of many cancers. The standard of care regimen is an IV formulation infused over 1 hour. The IV formulation includes polysorbate 80 which can lead to hypersensitivity reactions and peripheral neuropathy. Oral Docetaxel plus Encequidar (oDox+E) is a novel oral docetaxel regimen which consists of oral docetaxel and 30mg of Encequidar, a novel gut specific P-GP inhibitor, given 1 hour before Oral Docetaxel.

Aim: To establish and explore Go / No-Go criteria for oDox+E.

Methods. A phase I dose escalation trial of oDox+E was carried out in patients with metastatic prostate cancer who were prescribed IV docetaxel. Nine patients in total completed the study and provided total and unbound concentration data after both IV docetaxel and oDox+E at various dosing regimens. A population pharmacokinetic model was developed, and 1000 virtual patients were simulated from this model. The area over the minimum effective concentration (MEC) was chosen as the exposure metric of interest. The percentage target attainment (PTA) was calculated for various oDox+E dosing regimens and various MEC values. Success was determined when the area under curve over the MEC for oDox+E was greater than or equal to 80% of the IV docetaxel value (dose based on standard of care). The proposed Go / No-go decision framework was: 1) Go if all regimens achieved a PTA of >80%, 2) conditional Go if some regimens achieved a PTA of >80%, and 3) No-Go if no regimens achieved a PTA of >80%.

Results. A 3-compartment population pharmacokinetic model was developed for IV docetaxel and oDox+E in NONMEM. The figure summarises the PTAs for three doses of oDox+E, some dosing regimens achieved a PTA of >80%. Therefore, a conditional Go decision was proposed.

Discussion. 600mg of oDox+E given 3 times achieved non-inferior exposure compared to the current standard of care IV docetaxel regimen. A conditional GO decision was proposed with the recommendation to further characterise the MEC value prior to Phase IIa/b trials.

A19

Co-expression of CGRP, amylin and the CTR in the dorsal root ganglia and spinal cord of mice, rats and humans

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Introduction. Calcitonin-gene related peptide (CGRP) and amylin are closely related peptides which have been linked to pain. These peptides are potent agonists of the AMY_1 receptor, a heterodimer of the calcitonin receptor (CTR) and RAMP1. Expression of both peptides has been reported in pain relevant neural structures, including the dorsal root ganglia (DRG) and spinal cord (SC). This suggests that the AMY_1 receptor may be involved in pain. Interestingly, CTR protein expression has not yet been examined in either rat or human DRG and SC, and there are only limited reports in the DRG and SC of mice. Overall, the exact localisation of CTR in these regions and whether it is expressed together with its agonists is poorly understood.

Aims. To determine the relative distribution of AMY_1 receptor component, CTR, with CGRP and amylin in the DRG and SC.

Methods. To study the distribution of CGRP, amylin and the CTR, thoroughly validated antibodies were used. Specific antibodies were applied to fixed mouse, rat and human DRG and SC sections, in combination with neural markers to investigate distribution.

Results. In the DRG, CGRP-like, amylin-like and CTR-like immunoreactivity (LI) was observed in the neuronal cell bodies of all species. CGRP-LI and amylin-LI was present in distinct and overlapping neurons, indicating occasional co-expression of the peptides. CTR-LI was present in neurons which expressed CGRP or amylin alone, as well as neurons which expressed CGRP and amylin together. Co-staining with NF200, an A-fibre marker, indicated that CTR-LI, CGRP-LI and amylin-LI was likely to be in C-fibre neurons. In the SC, CTR-LI, CGRP-LI and amylin-LI was observed in the dorsal and ventral horns of all species. In the dorsal horn, CGRP and amylin were localised to fibres in laminae I-II, which did not express NF200, whereas CTR-LI was present in small neuronal cell bodies. In the ventral horn, immunoreactivity of all three proteins was observed in motor neuron-like cells.

Discussion. The data suggest that CGRP and amylin could play both individual or cooperative roles in pain transmission in the peripheral and central nervous system via the AMY_1 receptor in C-fibre neurons, rather than A-fibre neurons.

A20

Cellular uptake of clozapine and its major metabolites.

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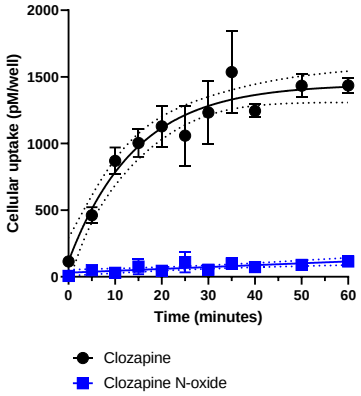

Introduction. Clozapine (CLZ) is the gold standard therapy for treatment resistant schizophrenia, although is associated with serious adverse drug reactions such as myocarditis[1]. While mechanisms are unknown, one proposed hypothesis suggests CLZ or one or more primary metabolites, *N*-desmethylclozapine (DMCLZ) or clozapine-*N*-oxide (CLZNO), may be directly toxic to cardiac tissue. Previous research suggesting a role for *N*-oxide metabolites in idiosyncratic drug reactions is supportive of this hypothesis[2] and we have found that myocarditis patients had a higher CLZNO:DMCLZ ratio in plasma than non-cases (manuscript in preparation). Knowledge of how CLZ, DMCLZ and CLZNO enter tissues is therefore important in elucidating mechanisms of clozapine toxicity, although current knowledge of CLZ and metabolite uptake into cells is minimal.

Aims. To determine cellular transport mechanisms of CLZ, DMCLZ and CLZNO.

Methods. Initial investigations of clozapine cellular transport were undertaken in Caco-2 due to their wide transporter expression. Cells were seeded in 24 well plates and left to adhere for 24 hours (37°C in 5% CO_2) prior to incubation with ^{3}H-CLZ and ^{3}H-CLZNO (0.3 – 15 µM) for up to 60 minutes. Post incubation, Caco-2 cells were washed with ice cold buffer, lysed, and analysed by scintillation (^{3}H DPM).

Results. Uptake of CLZ (3 µM) was saturable ($t_{1/2}$ = 9.9 min) and plateaued at 1446 pM/well. CLZNO accumulation was minimal (5.3% ± 1.3% of clozapine uptake) and linear over the 60 minute incubation.

Discussion. CLZ cellular uptake is consistent with transporter mediated-processes in CaCo-2. In contrast, CLZNO accumulation was limited, possibly due to either a lack of uptake transporter affinity or to efficient efflux of this compound, requiring further studies to determine specific transporter involvement.

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2. Uetrecht, J. N-oxidation of drugs associated with idiosyncratic drug reactions. *Drug Metab Rev* **34**, 651–665 (2002).

A21

A new hope: Calcitonin gene-related peptide blocking drugs in migraine

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Migraine is a complex, disabling primary headache disorder that is highly prevalent. Many people living with migraine have no effective treatment. Migraine affects day-to-day life and is the 2nd highest cause of years lived with disability worldwide. Calcitonin gene-related peptide (CGRP), a neuropeptide, is expressed in sensory neurons and is a known migraine trigger. This presentation will outline the recent success in developing CGRP blocking drugs for the treatment and prevention of migraine, giving those living with migraine an important new treatment option. The presentation will cover the rationale for targeting CGRP in migraine, outline the mechanisms of action of CGRP blocking drugs, highlight some of the opportunities and challenges that remain, and consider future directions.

A22

Prophylactic organic carbon monoxide donors protect hypertrophic hearts against ischaemia-reperfusion injury.

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Introduction. Diminished cardiac output resulting from ischaemia-reperfusion (I/R) injury and peri-operative complications, are commonly present in cardiac surgical interventions involving cardiopulmonary bypass. Pre-existing cardiac pathologies, such as hypertrophic cardiomyopathy potentiate these peri-operative complications and result in reduced outcome benefits. A new class of carbon monoxide delivery molecules (oCOms) have been developed as potential cardioprotective agents. The present study investigated the pharmacological effect of oCOm-21 in hypertrophic hearts subjected to an acute I/R episode and further assessed the immediate *in-vivo* effects of oCOm-21.

Aims. 1. To compare the prophylactic benefit of pre-ischaemic treatment with oCOm-21 in normotrophic and in hypertrophic rat hearts in an ex-vivo model of I/R injury. 2. To assess the *in-vivo* effects of oCOm-21 following intravenous bolus infusion.

Methods. In aim 1, hypertension induced in male Cyp1a1-Ren2 rats fed indole-3-carbinol (0.167%; eight-weeks) resulted in cardiac hypertrophy and fibrosis ($P < 0.001$) against control littermates. Hearts were isolated and perfused using the Langendorff technique. oCOm-21 (1 - 10 µM) or vehicle control was infused (10-minutes) prior to a 30-minute warm global ischaemic episode followed by a 60-minute reperfusion period. In aim 2, male Sprague-Dawley rats (320 – 350g) were administered oCOm-21 (5 - 25 □mol/kg) or vehicle control intravenously with mean arterial blood pressure and carboxyhaemoglobin levels assessed over a 2 hour period.

Results. In normotrophic hearts ($n = 5$/group), oCOm-21 (1 & 3 µM) improved left ventricular developed pressure (LVDP) recovery ($P < 0.01$ & $P < 0.001$ respectively against vehicle control). In hypertrophic hearts ($n = 8$ - 10/group), LVDP recovery to pre-ischaemic baselines was significantly improved with 3 and 10 µM oCOm-21 compared to control. Intravenous oCOm-21 (10 & 25 □mol/kg) administration ($n = 5$/group) resulted in an immediate and significant reduction in mean arterial blood pressure ($P < 0.01$ & $P < 0.001$ respectively against vehicle control). Blood carboxyhaemoglobin levels over the 2 hour recording period did not exceed 5% at any of the administred oCOm-21 concentrations.

Discussion. This study provides valuable evidence supporting the safe use of oCOm-21 as an effective pre-conditioning agent in acute cardiovascular interventions in hearts burdened with hypertrophic cardiomyopathy.

A23

Carbon Monoxide Release by oCOm-21 Enhances Ca^{2+} Sensitivity of the Cardiac Myofilament

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Introduction. Inotropic therapies increase contractile force by enhancing cytosolic Ca^{2+} cycling. However, elevated Ca^{2+} can adversely increase myocardial oxygen demand, leading to arrythmias, prompting a demand for improved inotropes which do not affect intracellular Ca^{2+} cycling. We have demonstrated that carbon monoxide (CO) concentrations (1 - 10 μM) delivered by the prodrug oCOm-21, an organic CO releasing molecule, evoke a concentration-dependent, inotropic effect within isolated perfused hearts. Although the positive inotropic mechanism of CO remains unclear, CO can interact with myofilament proteins such as troponin C which may increase Ca^{2+} sensitivity. While this action has not been examined in cardiomyocytes, we propose that the inotropic effect of oCOm-21-derived CO may result from a CO-mediated increase in cardiac myofilament Ca^{2+} sensitivity.

Aims. To identify whether CO release by oCOm-21 produce an inotropic response by directly increasing the Ca^{2+} sensitivity of the contractile filaments.

Methods. Rat left ventricular cardiomyocytes were skinned, isolated, and attached to a force transducer within a micro-organ bath at 15°C. Force was measured as a function of pCa (-log [Ca^{2+}]) in the range of pCa 9.0-4.5 under two conditions: vehicle control or oCOm-21 (10 mM) (9 cells/group). The Ca^{2+} concentration at which 50% of maximal force is produced (pCa_{50}), was derived from each condition and used to measure a change in Ca^{2+} sensitivity. Results were analysed using an unpaired Student's t-test.

Results. oCOm-21 at 10 mM induced a significant 10-fold increase in pCa_{50} compared to vehicle control respectively (pCa_{50} 5.52 vs. 5.43; $P < 0.01$). Additionally, oCOm-21 did not significantly affect the maximal force exerted by the cardiomyocytes versus vehicle (15.34 vs. 16.23 kN/m^2) ($P > 0.05$).

Discussion. These preliminary results support the hypothesis that CO release by oCOm-21 increases Ca^{2+} sensitivity of the cardiac contractile filament without increasing maximal force production. Further analyses will identify the exact interaction CO has with the contractile filament to exert this Ca^{2+} sensitising effect.

A24

Antibiotic pharmacodynamics: effect of exposure on renal function

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Introduction. Gentamicin, amikacin and vancomycin are antibiotics used to treat bacterial infections from neonates to adults. They have activity against multi-drug resistant bacteria, but are associated with nephrotoxicity. Safe and effective use of these antibiotics would be helped by understanding the exposure-response relationship with nephrotoxicity.

Aim. To develop a population pharmacodynamic model linking exposure of potentially nephrotoxic antibiotics to individual renal function (RF).

Methods. Antibiotic exposure (AUC) was calculated using the dose and individual Bayesian estimate of clearance from a population pharmacokinetic model. RF was calculated using the ratio of individual estimated creatinine clearance to individual normal glomerular filtration rate. It is a continuous metric normalised to size, maturation and body composition. RF was used as the dependent variable with antibiotic exposure as independent variable and postnatal age (PNA) and sex as covariates. Population parameter variability assumed a log normal distribution for the random effects except for Emax which assumed a logit distribution. Parameter estimation used NONMEM 7.5.1.

Results. The exposure-response relationship was best described using a sigmoid Emax model. Population parameter estimates are shown in the table. RF declined over time with PNA (binned by decade). There was no association of sex on RF.

Drug	Emax [95% CI]	AUC50 mg/L*h [95% CI]	HILL [95% CI]
Gentamicin	-0.460 [-0.497, -0.402]	69.0 [66.5, 71.4]	1.22 [1.10, 1.61]
Amikacin	-0.257 [-0.274, -0.226]	56.3 [39.0, 81.9]	2.81 [2.22, 4.62]
Vancomycin	-0.999 FIXED	404 [394, 428]	2.17 [1.97, 2.56]

Discussion. There was a marked decrease in renal function associated with AUC for gentamicin and vancomycin, but not so large with amikacin. The AUC50 is similar to the target AUC recommended for clinical benefit. This study quantifies the exposure-response relationship for nephrotoxicity and provides an estimate of the magnitude of renal toxicity associated with targets based on clinical benefit.

A25

Reactive Oxygen Species associated with CYP-catalysed Cycling of Clozapine to Clozapine-*N*-oxide

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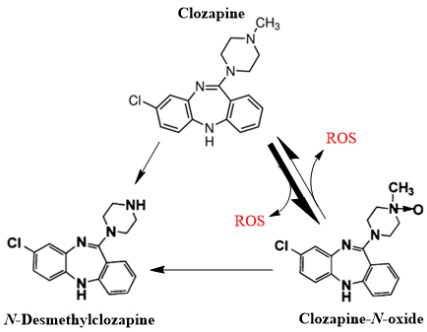

Introduction: Clozapine (CLZ) is an atypical antipsychotic, limited in its prescription due to the risk of adverse drug reactions. A primary circulating metabolite, *N*-desmethylclozapine (DMCLZ), is routinely monitored, however, the other major plasma metabolite, clozapine-*N*-oxide (CLZNO), is often ignored and under-investigated. We have found that myocarditis patients had a higher CLZNO: DMCLZ ratio than case controls (manuscript in preparation). Interconversion between CLZ and CLZNO has been attributed to a reversible metabolic process[1]. It has been postulated that this redox cycling produces reactive oxygen species (ROS) can damage cardiomyocytes[2], which has been detected in rat hearts following high doses of CLZ[3]. It is hypothesised that the cycling may increase the risk of CLZ-induced myocarditis.

Aim: To investigate the role of various CYP isoforms in the redox cycling of CLZ and CLZNO and related ROS formation.

Methods: Clozapine (or a primary metabolite) was incubated with individual CYP isoforms in the presence of 2'-7'-dichlorofluorescein diacetate (DCF-DA). DCF-DA oxidation to dichlorofluorescein was detected using spectrometry (485 nm excitation; 520 nm emission) as an indirect marker of ROS. Concentrations of CLZ and metabolites were measured using a validated liquid-chromatography mass spectrometry method established within our laboratory[4].

Results: CYP isoforms that favour the CLZNO metabolic pathway were associated with significant ROS generation in a concentration-dependent manner. In contrast, the formation of DMCLZ did not correlate with ROS generation.

Discussion: Favouring the CLZNO metabolic pathway is consistent with increased plasma concentrations of CLZNO in cases of clozapine-induced myocarditis relative to non-cases[4], indicating that these CYP may contribute to the development of cardiotoxicity by instigating a pro-inflammatory state in cardiac tissue.

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2. Tada, Y. & Suzuki, J.-I. Oxidative stress and myocarditis. *Curr Pharm Des* **22**, 450–471 (2016).
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A26

Sevoflurane pharmacokinetics and pharmacodynamics in adults during general anaesthesia

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Introduction. The relationship between arterial sevoflurane concentration and depth of anaesthesia is poorly described. Published physiological models often do not consider co-administered drugs on anaesthetic effect nor investigate the influence of body composition on sevoflurane pharmacokinetics.

Aims. We aimed to quantify the relationship between inspired sevoflurane concentration, arterial concentration and bispectral index in adults using compartmental models.

Methods. Sevoflurane dose, arterial sevoflurane concentration and bispectral index were analysed using nonlinear mixed effects models. Total body weight, normal fat mass and fat-free mass were investigated as covariates to describe differences in sevoflurane pharmacokinetics between individuals. Participants received remifentanil (effect-site target 3-5 µg/L) and propofol was given at the discretion of the anaesthetist. Sevoflurane pharmacokinetics were described using a three-compartment model. Bispectral index was described using a sigmoidal effect (E_{MAX}) model. An effect-site compartment was used to relate sevoflurane concentrations to effect, linked using an equilibration half-time, $T_{1/2}$ keo.

Results. Participants were 60 adults (19-87 years, 44-160 kg) with sevoflurane as their primary anaesthetic agent. The parameter estimates (%CV) were: CL 621 (55.1%) L/h/70 kg, Q2 619 (88.4%) L/h/70 kg, Q3 590 (29.2%) L/h/70 kg, V1 32.6 (59.7%) L/70 kg, V2 108 (55.2%) L/70 kg, V3 6030 L/70 kg. Baseline bispectral index (E_0) was 98.5 (72.5%) and E_{MAX} was 0.63 (33.3%). The effect-site concentration producing half of E_{MAX} (Ce_{50}) was 17.7 (19%) mg/L and the Hill exponent was 5.8 (63.9%). The effect compartment $T_{1/2}$ was 1.1 minutes. The Ce_{50} for propofol and remifentanil were 1.78 mg/L (12.4%) and 11.9 (9.4%) µg/L, respectively. The Greco response-surface model described the additive effects of propofol and remifentanil with sevoflurane on bispectral index.

Discussion. Sevoflurane pharmacokinetics were best described by total body weight. The short effect compartment $T_{1/2}$ suggests rapid distribution of sevoflurane from the blood to the effect-site.

A27

Combination of ALK and SHP2 inhibitors produce synergistic suppression of ALK-positive lung cancer cell growth

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Introduction. Lung cancer is the leading cause of cancer mortality worldwide, accounting for approximately 1.6 million deaths per annum. The most common form of lung cancer is non-small cell lung cancer, of which anaplastic lymphoma kinase (ALK) mutations occur in approximately 6% of cases. The second-generation ALK inhibitor, alectinib, has markedly prolonged ALK-positive cancer patient survival. However, patients rarely survive more than 2-3 years. One proposed strategy for improving drug efficacy is by co-targeting secondary oncogenic drivers. Here we focus on SHP2. However, as SHP2 is expressed ubiquitously used as a monotherapy, SHP2 inhibitors are likely to be considerably dose limited by toxicity. One strategy for reducing toxicity is to reduce the minimum effective dose by administering the drug in combination with another to produce synergistic efficacy.

Aims. Establish whether the addition of SHP099 (SHP2 inhibitor) to alectinib increases the efficacy of alectinib and decrease SHP099 toxicity in ALK-positive non-small cell lung cancer cells and the mechanism by which this occurs.

Methods. The sulforhodamine B assay was used to assess the cell viability of H3122 cells following a 72-hour incubation with alectinib, SHP099, or the combination. Synergy was examined using the Bliss combination index. Western blotting was used to assess the expression of downstream signalling pathways and apoptotic and cell cycle markers in alectinib, SHP099, and combination treatment groups to determine the mechanism associated with the synergy.

Results. Our results demonstrated that the combination significantly and synergistically decreased the cell viability at low concentrations ($p < 0.05$) and decreased downstream signalling pathways. The combination also resulted in a significant G1-phase cell cycle arrest associated with a reduction in cyclin D1. The pro-apoptotic markers, Bim, and cleaved caspase-3 were also significantly increased with the combination compared to alectinib ($p < 0.05$).

Discussion. These preliminary results support the hypothesis that the addition of SHP099 to alectinib produces a synergistic effect to increase the efficacy of alectinib through G1 cell cycle arrest and induction of intrinsic apoptosis. Future *in vivo* experiments will explore the combination in a mouse tumour model to examine the efficacy and toxicity.

A28

Tumour cell suppression by Spiroleucettadine through dual regulation of cell cycle and apoptosis.

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Introduction. (-)-Spiroleucettadine is an alkaloid originally derived from the yellow Leucetta sea sponge. Spiroleucettadine has previously been shown to inhibit the growth of various cancer cell lines, in particular high anti-proliferative activity against a non-small cell lung cancer cell line (H522). The mediators of these anti-proliferative effects have not been determined.

Aims. In this study we measured changes in cell death and cell proliferation and their immediate protein mediators in response to spiroleucettadine, toward the aim of ultimately determining target(s) for spiroleucettadine in cancer cells and a more precise description of its mechanism of action.

Methods. The cytotoxicity of spiroleucettadine was examined in p53 mutated NSCLC (H522) cells using the SRB assay. Cell cycle arrest and apoptotic induction of H522 cells by spiroleucettadine was measured using flow cytometry analysis. Western blotting was undertaken to determine changes in expression of key cell cycle and apoptotic proteins following spiroleucettadine treatment.

Results. We found evidence for cell cycle arrest at G1/M and associated increases in cyclin B1 expression and CDK1 phosphorylation, as well as an increase in apoptosis alongside marked increase in Bim expression, consistent with activation of the intrinsic apoptotic pathway.

Discussion. Spiroleucettadine inhibited cell proliferation, produced a G2/M phase cell cycle arrest and induced apoptosis at low micromolar concentrations. G2/M cell cycle arrest was achieved via increased pCDK1 expression while apoptosis was induced through increased Bim expression leading to activation of cleaved caspase 3. Further studies should investigate the potential action of spiroleucettadine on microtubules and *in vivo* activity to further determine the suitability of spiroleucettadine for clinical lung cancer treatment.

A29

Positive allosteric modulation of the type 1 cannabinoid receptor potentiates endocannabinoid signalling and changes ERK1/2 phosphorylation kinetics.

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Introduction. Orthosteric activation of CB_1 by exogenous agonists is known to cause a plethora of adverse side effects *in vivo*. Positive allosteric modulation is an interesting therapeutic approach that is hoped to offer improved therapeutic potential (antinociception/reducing intraocular pressure, among other indications) and a reduced on-target side effect profile compared to orthosteric agonists. Endocannabinoids 2-AG and AEA orthosterically activate CB_1 under physiological conditions, and it is not clear how positive allosteric modulators would affect this paradigm.

Aims. To characterise the *in vitro* signalling profile of PAMs alone, and in combination with the endocannabinoids 2-AG and AEA, and a synthetic cannabinoid AMB-FUBINACA, with a focus on receptor regulation.

Methods. Bioluminescence resonance energy transfer assays in HEK293 cells were preformed to investigate G protein dissociation, ERK1/2 phosphorylation and β-arrestin 2 translocation paradigms, while immunocytochemistry was performed to measure internalisation of CB_1 in response to the PAMs ZCZ011, GAT229, and ABD1236 alone and in combination with orthosteric agonists.

Results. All PAMs were found to be allosteric agonists in all pathways tested. In combination with orthosteric agonist they increase the potency and efficacy of AEA-induced G protein dissociation, while only increasing efficacy of G protein dissociation caused by 2-AG and AMB-FUBINACA. Interestingly, the PAM ZCZ011 (alone) induced a different ERK1/2 phosphorylation time course compared to that of orthosteric agonists, entailing a biphasic response (contrasting to a more classical single, transient activation). In combination with 2-AG, all PAMs caused the transient peak of ERK1/2 phosphorylation to become a sustained response. In combinations with orthosteric agonist all PAMs caused an increase in potency and efficacy of AEA-induced β-arrestin 2 translocation, while only increasing potency of 2-AG and AMB-FUBINACA-induced β-arrestin 2 translocation.

Discussion. PAMs can potentiate endocannabinoid CB_1 agonism, so their effects may retain the physiologically-regulated spatiotemporal signalling of the endocannabinoids. Compared to the global receptor activation following exogenous orthosteric agonist administration, this may underpin the more favourable adverse effect profile of PAMs.

A30

Optimal insulin initiation dose in children with type 1 diabetes mellitus

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Introduction. Insulin dose requirements vary between patients with type 1 diabetes mellitus (T1DM). Bolus doses depend on the patient's insulin to carbohydrate ratio (ICR), for meal-time dosing, and on the correction factor (CF), for elevated blood glucose correction. In clinical practice, it usually takes several days of heuristic insulin dose adjustments, guided by blood glucose concentration measurements and carbohydrate counting before a patient's ICR and CF values can be accurately estimated.

Aims. (1) To review commonly used ICR and CF estimation models, and (2) to develop and evaluate new models for ICR and CF using local (Southern DHB) patient data.

Methods. 1) A scoping literature review of online databases and search engines was conducted to identify commonly used ICR and CF formulae. 2) Linear regression analyses of retrospectively collected data was undertaken using clinical characteristic, laboratory measurements, and insulin dosing data from 70 children (≤ 18 years old) admitted to hospital due to new onset T1DM. Regression models for ICR and CF values were developed and evaluated for bias and precision using mean error and root mean squared error, respectively.

Results. 1) According to the literature, covariates such as age and weight are commonly used to estimate ICR and CF.
2) Based on the final ICR and CF prediction models, for patients < 6 years old, total daily dose of insulin administered on first day () was the only significant predictor for ICR and CF ($p < 0.05$). For patients ≥ 6 years old, patient and were found to be significant predictors for both ICR and CF ($p < 0.05$).

Discussion. Accounting for a patient's age and insulin allows for better estimation of true (i.e. asymptotic) ICR and CF values. This can potentially provide better glycaemic control sooner during hospital stay.

A31

Adverse Drug Reactions in Older Adults

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A32

Extracellular bimolecular fluorescence complementation assays for investigating receptor dimerization: a proof of concept with receptors for calcitonin gene-related peptide.

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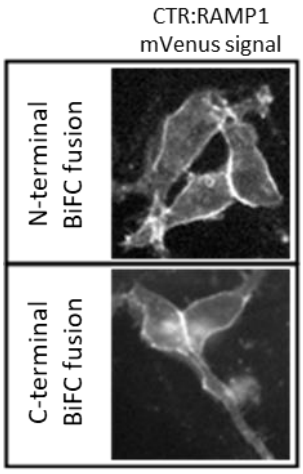

Introduction. Bimolecular fluorescence complementation (BiFC) methodology utilises a fluorescent protein split in two to detect protein:protein interactions in living cells. BiFC can be used to study G protein-coupled receptor (GPCR) dimerization. To date, split fluorescent proteins have only been fused to the intracellular C-termini of GPCRs. Fusing these to the extracellular GPCR N-terminus may improve the flexibility of this methodology and reduce the impact on signal transduction. As a proof of concept, we used receptors for calcitonin gene-related peptide. These comprise heterodimers of either the calcitonin receptor (CTR) or calcitonin receptor-like receptor (CLR) in complex with an accessory protein (receptor activity-modifying protein 1; RAMP1).

Aims. To compare N-terminal fusion constructs to C-terminal fusion constructs in assays measuring ligand stimulated signalling (cAMP production), cell-surface expression of receptor complexes, and fluorescence yields of BiFC proteins.

Methods. Split mVenus fragments were attached to either the C-termini or N-termini of receptors using InFusion methodologies. Resulting constructs were transiently transfected into HEK293S and Cos7 cells. Receptor activation was assayed by measuring cAMP production. Cell surface expression and BiFC fluorescence were measured by high-content imaging.

Results. N-terminal fusions had a lesser impact on cAMP signalling than C-terminal fusions. Both C-terminal and N-terminal fusions created functional fluorescent proteins, though N-terminal fusions had relatively lower fluorescence than C-terminal fusions.

Discussion. Our results suggest that BiFC methodology can be successfully targeted to the N-terminus of GPCRs, increasing the flexibility of this approach and enabling further insights into GPCR dimerization.

A33

Investigating the signalling of an atypical group of receptors – GPCR-bigrams

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Introduction. *Phytophthora agathidicida* is the plant pathogen responsible for kauri dieback disease. This soil dwelling oomycete uses G protein-coupled receptors (GPCRs) to detect and respond to compounds released from kauri roots. This suggests that classical human drug discovery approaches for targeting GPCR may be useful to target this pathogen. *P. agathidicida* expresses GPCRs with typical topologies as well as numerous atypical GPCRs that have various catalytic domains fused to the 7-transmembrane helix bundle of the receptor. These atypical receptors are collectively referred to as GPCR-bigrams, and their function remains very poorly understood.

Aims. To determine whether GPCR-bigrams signal using classical G protein signalling pathways, and/or directly modulate the respective catalytic fusion domains phosphatidylinositol phosphate kinase (PIPK), inositol polyphosphate phosphatase (INPP), tyrosine-kinase like (TKL), and adenylate cyclase (AC).

Methods. As no ligands have been identified for any GPCR-bigram, chimeric receptors were generated; the 7TMH domains of the well characterised receptors β2-AR and CHRM1 were fused to a *P. agathidicida* PIPK, INPP, and TKL, or AC, respectively. Real time BRET biosensor assays, including three novel biosensors to detect the activity of the PIPK and INPP domains, were used to specifically detect cAMP, PIP, PIP_2, or PIP_3 levels in mammalian cells.

Results. We have shown the three β2-AR chimeric receptors can still signal via classical G-protein signalling; however, the CHRM1-AC chimera does not appear to maintain G_q linked signalling. The potency of isoprenaline is reduced by ~1 log unit for the β2-AR chimeras vs wild-type β2-AR. Our new BRET biosensors can detect changes in PIP, PIP_2, or PIP_3 levels (the substrates or products of PIPK and INPP enzymes) in response to activation of a G_q linked receptor. To date, we have been unable to detect direct modulation of the fused catalytic domains in response to GPCR activation.

Discussion. GPCR-bigrams are an atypical group of GPCRs present in many single celled eukaryotes. These receptors are likely involved in pathogenesis, however, almost nothing is known about their signalling mechanisms. Our work highlights that it is still possible for some GPCRs with C-terminal catalytic domain fusions to signal via heterotrimeric G proteins. Further work is required to determine if the catalytic PIPK, INPP, TKL, and AC domains can be directly modulated via the GPCR domain, or whether these domain fusions play a different role in cellular signalling.

A34

A survey of 6-thioguanine use by gastroenterology clinicians for inflammatory bowel disease

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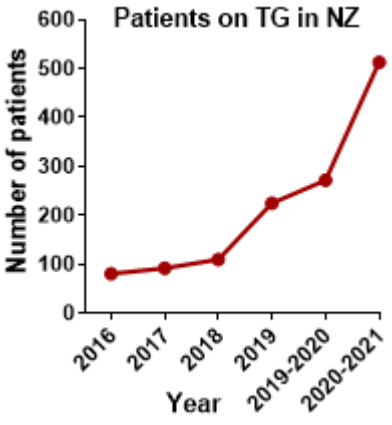
Introduction. The thiopurines remain mainstay treatments in autoimmune diseases especially inflammatory bowel disease. However azathioprine and 6-mercaptopurine are not tolerated in 30% and are ineffective in 20% of patients. There is growing evidence that thioguanine (TG) is safer and more effective than conventional thiopurines. Review of PHARMAC prescribing data shows an exponential increase in TG use in NZ with >500 patients now on this treatment.



Aims. We conducted an on-line survey to assess use of thioguanine (TG) by gastroenterologists in NZ for IBD, including usage, dosing and monitoring.

Methods. Gastroenterologists and trainees were invited to complete a 14-question on-line Google survey during 2020.

Results. There were 77 respondents. At the time of the survey, 48% of respondents were using TG, of which 11.5% were using this as their first-line thiopurine. 20mg daily was the typical dose in 92% of responses. Most clinicians had used TG in less than 10 patients but 2 clinicians had used TG in 35 and 50 patients. 65% of clinicians were using TG in combination with a biologic and 35% as monotherapy. 89% of clinicians using TG were monitoring metabolite concentrations but the chosen therapeutic range for 6-TGN was variable, with lower to upper limits ranging from 235 to 1200 pmol/8x10^8 RBCs. TG was stopped in 11 patients due to adverse effects, which were similar to those seen with conventional thiopurines.

Discussion. Use of TG for IBD is growing rapidly in New Zealand due to increasing confidence in this therapy. Most clinicians are using TG appropriately regarding dose and monitoring but in 2020 there was some confusion regarding the target range for the concentration of the 6-thioguanine nucleotide metabolites.