400 A Retrospective, Longitudinal Study of Antibiotic Prescribing at a Western Australian Hospital.

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Introduction: The rising incidence of antimicrobial resistance due to inappropriate antibacterial agent prescribing and usage has significantly increased patient morbidity, mortality, and the associated societal and healthcare costs. In Australian hospitals, an annual National Antibacterial Prescribing Survey (NAPS) provides data on indications and the appropriateness of antibacterial agent prescribing.
Methods: Census data of 1051 prescriptions from 2013 to 2017 were used to investigate the most common indications for prescribing antibiotics and the appropriateness of prescribing.
Results: The most common indications included surgical prophylaxis (128; 12.2%), community acquired pneumonia (CAP) (82; 7.8%) and skin and soft tissue infections (SSTI) (53; 5.0%). The highest frequency of antibacterial prescribing occurred in three specialities; general surgery (139/1051; 13.2%) where 49.6% of prescriptions were non-compliant with guidelines and 28.8% were inappropriate, plastic surgery (128/1051; 12.2%) (55.5% non-compliant, 50.0% inappropriate); and general medicine (123/1051; 11.7%) (39.0% non-compliant, 30.9% inappropriate). Non-compliant (90.6%) and inappropriate (86.7%) prescribing was high in surgical prophylaxis, but lower for CAP (58.3% and 43.9% respectively) and SSTI (52.8% and 24.5% respectively). Antibiotics most commonly prescribed for surgical prophylaxis included cefazolin (64.1%) and cefalexin (8.6%), for CAP, azithromycin (29.3%) and ceftriaxone (29.3%) and for SSTI, flucloxacillin (45.3%) and cefazolin (18.9%).
Discussion. The high rates of non-compliant and inappropriate antibacterial prescribing identified, especially in surgical prophylaxis, were consistent with national Australian, and global trends, highlighting the need for improved antimicrobial stewardship (AMS) to increase guideline compliance.

401 Enhancing guideline implementability through communication design strategies: a systematic scoping review

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Introduction. Clinical guidelines are a synthesis of evidence-based recommendations that are used in clinical settings to mitigate decision variability and standardise care. Guideline uptake in practice is low, despite many interventions trialled to improve overall implementability.
Aims. To identify interventions used to improve guideline implementability via optimisation of communication design, and to report on the outcomes of such interventions.
Methods. A scoping review was conducted using a systematic search strategy, which was developed using a combination of two key concept groups: i) clinical guidelines (clinical guidelines, practice guidelines); and ii) implementability (design, format, communication, language). The search was conducted across four databases: Medline, Embase, Scopus, and CINAHL. Communication design strategies were mapped according to the domains of message and format as outlined by Kastner and colleagues (Kastner et al., 2015).
Results. A total of 5938 reports were screened and citation chaining was performed, producing 29 included reports. A variety of communication design interventions were identified that aimed to improve the implementability of guidelines toward the user, and for integration into computer systems. Most interventions focused on enhancing computer implementability of guidelines (19, 65.5%), or the inclusion of guidelines in clinical decision support systems (11, 37.9%). The remaining studies looked at modelling guidelines using computer-interpretable language (8, 27.6%). Other strategies examined included enhancing user implementability through guideline reformatting (2, 6.9%), and the development of smartphone applications (2, 6.9%). All but two studies demonstrated improved guideline adherence and implementability following the use of communication design strategies.
Discussion. Whilst a variety of strategies aimed to improve the implementability of guidelines by optimising guideline design, the majority of studies centred around enhancing implementability through electronic systems. Most studies reported improved adherence and usability of guidelines following their intervention, however, the sustainability of interventions was not evaluated.

402 Co-designing the PRIME tool

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Introduction. The process of shared decision-making about deprescribing (reducing or stopping potentially inappropriate medications) between people living with dementia and their carers (consumers), and healthcare professionals (HCPs), is complex.
Aims. To co-design a consumer-centric, educational tool to empower people living with dementia and their cares to initiate a deprescribing conversation with their HCP.
Methods. We formed a stakeholder steering group (SG) consisting of 6 consumers and HCPs (two geriatricians, a general medicine clinician, a nurse practitioner, and a social worker) from Australia and the United States. Five one-hour SG meetings were held. These involved: 1) Introductions and familiarisation; 2 & 3) selecting key elements and co-designing the PRIME tool (using a baseline draft tool based on an existing validated patient questionnaire and previous research works of members of the research team); 4) pilot testing an interview guide to test the usability and comprehensibility of the PRIME tool; and 5) co-designing the implementation of the PRIME tool in real-life practice.
Results. We have co-designed a draft of the PRIME tool which consists of three main sections (background; reflection; call to action). Each section consists of various key elements selected by the SG. For example, consumers suggested including “key phrases” to prompt a deprescribing conversation and including designated spaces to reflect and write down medicines they would like to discuss with their HCP. With feedback from the SG, we have also planned early steps to test the implementation of the tool in real-life practice.
Discussion. The PRIME tool draft was co-designed with comprehensive involvement of an international stakeholder SG. Consumers using the PRIME tool may be more empowered to initiate deprescribing conversations with their HCP. Future studies will involve further refining and pilot testing of the tool in real-life clinical practice.

403 Patient characteristics and experiences with pharmacy immunisation services in New Zealand

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Introduction. New Zealand pharmacists have been providing immunisation services since 2011. Literature from other developed countries reports the positive experience of people with community pharmacy immunisation services resulting in expansion of the scope of pharmacy practice. However, there is a dearth of such data in a New Zealand context.
Aims. To understand patients’ experiences and perceptions surrounding being vaccinated by a pharmacist.
Methods. A self-administered questionnaire was developed after considering the aims and objectives of the study, and previously published literature. The survey assessed patients’ experiences in a community pharmacy setting and measured their satisfaction using a 5-point Likert scale. Fourteen pharmacies providing immunisation services and covering a range of socioeconomic areas across New Zealand were identified to help with data collection.
Results. Out of the 364 survey participants, 60.7% were female, 76.9% were of European ethnicity, and 43.4% belonged to the age group of 45-64 years. Convenience (65.4%) and accessibility (44.8%) were cited as the most common reasons for choosing a community pharmacy to get vaccinated. Over 90% of the respondents reported that they were satisfied with the overall experience of being vaccinated at a pharmacy, that they were vaccinated professionally, that they will choose community pharmacy again next time for vaccination, and that they would like to see pharmacists administering other vaccines**.**
Discussion. The overall positive response found in our study suggests that pharmacists administering the influenza vaccine are highly valued by patients, as well as improving access to immunisation, and potentially increasing vaccination rates. Our findings suggest that expanding vaccination services of pharmacists to include a range of other common vaccines, including child vaccinations, would likely receive a positive reception by patients, and in turn lead to improvement in overall vaccination rates in New Zealand

404 Impact of partnered pharmacist medication charting on potentially inappropriate medication use

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Introduction. With the aim of improving the quality use of medicines and enhancing interdisciplinary collaboration, a partnered pharmacist medication charting (PPMC) model was trialled in the emergency department (ED) of a tertiary hospital.
Aims. To evaluate the impact of PPMC on potentially inappropriate medication (PIM) use.
Methods. A controlled study compared PPMC to two comparison groups among older people (≥ 65 years) presenting to ED. In the PPMC group, pharmacists initially obtained the best-possible medication history (BPMH) and collaborated with medical officers to co-develop treatment plans and chart medications. The early BPMH group included BPMH taking by pharmacists, followed by traditional medication charting by medical officers. The usual care group followed the traditional charting approach by medical officers, without a pharmacist-collected BPMH. Using Beers criteria, PIM use was assessed at ED presentation (i.e. at baseline), ED departure, and hospital discharge. Between-group comparisons used the Kruskal-Wallis test with Dunn's post-hoc test. Within-group comparisons (on ED departure/hospital discharge vs at baseline) used the Friedman rank sum test with a pairwise Wilcoxon rank-sum post-hoc test or Cochran's Q test with Dunn's post-hoc test, as appropriate.
Results. The use of at least one PIM on ED departure was significantly lower for the PPMC group than for the comparison groups (p=0.040). The results were also significant for the median number of PIMs per patient (p=0.036) and PIMs per prescribed medication (p=0.029) on ED departure. However, outcomes at hospital discharge were not statistically different. PIM use on ED departure or hospital discharge did not differ from baseline within the comparison groups.
Discussion. PIM use on leaving ED, but not at hospital discharge, was significantly reduced with PPMC.

405 Telepharmacy: the way of the future?

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Introduction. Longer travelling times, higher fuel costs, fewer General Practitioner (GP) visits and limited pharmacy services increase the health burden of those living in rural and regional areas.(1) These pressures are transferred to emergency department (ED) presentations that in turn experience access block, extended waiting times and overcrowding. One potential solution is telepharmacy. Telepharmacy, defined as "the provision of pharmacist care by registered pharmacists and pharmacies through the use of telecommunications to patients located at a distance".(2) We will evaluate a new telepharmacy service called The Pharmacist After Hours Advice Line (PAAL) Service.
Aims. To identify characteristics of users of the telepharmacy service. To understand the user's perspective of telepharmacy in terms of accessibility, acceptability, effectiveness, and experience of service users.
Methods. Service users will provide qualitative and quantitative feedback on accessibility, acceptability, effectiveness, experience, and demographics using a non-identifiable online survey.
Results. Data collection will begin in September 2022 and will be reported at the conference.
Discussion. Approximately 28% of Australians live in rural areas; they experience higher rates of hospitalisations, deaths, injury and poorer access to, and use of, primary health care services, than people living in major cities. Tasmania has one of the most rural and remotely dispersed populations of any state or territory, with just ten percent living outside the major population centres of Hobart, Launceston, Burnie and Devonport. There is a clear need to offer alternatives models of care that overcome the issues of cost, isolation, workforce availability and medication harm. The Pharmacist After Hours Advice Line will service rural Australians in Tasmania and will be targeted at those most at risk of medication harm including members of the general public, aged care facility staff and palliative carers.

1. Australian Institute of Health and Welfare. Rural and Remote Health [Internet]. Canberra ACT. 7 July 2022. Available from: https://www.aihw.gov.au/reports/rural-remote-australians/rural-and-remote-health
2. Win A.Z. Telepharmacy: Time to pick up the line. Res. Social Adm. Pharm. 2017;13:882–883. doi: 10.1016/j.sapharm.2015.06.002.

406 Health and medication related goals of care of older people with polypharmacy

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Introduction. Polypharmacy (5 or more medications) is associated with poor outcomes in older people (≥65 years), such as increased risk of adverse drug reactions (ADRs). Emerging evidence suggests that goals of care for older people can inform and optimise medication management, however these conversations are often sidelined with greater focus on disease state management.
Aim. To explore the goals of care of older people with polypharmacy with respect to overall health and medications.
Methods. Cross-sectional study within two settings: a) hospitalised older people or their carers were administered a questionnaire/ interviewed to identify their health and medication related goals of care; b) the same questionnaire was distributed to community-dwelling older people through advertisements in e-Newsletters of consumer groups (e.g., Older Person's Advocacy Network and Australian Dementia Network). Qualitative data from the goals of care questions were content analysed, and quantitative data from both studies were analysed descriptively.
Results. To date, data has been collected for n=21 hospitalised patients and n=20 community-dwelling older people, mean (SD) age 77.3 (6.9), and 74.3 (7.8), respectively. Preliminary content analysis of goals of care of hospitalised older people (n=17) and their carers (n=2) have identified health-related goals themes focusing on maintaining independence, increasing mobility, and increasing quality of life. Medication related goals of care themes encompassed ensuring medication adherence, reducing medication burden where possible, optimising therapy to better manage existing comorbidities and reducing ADRs. Health related goals of care themes of community-dwelling older people (n=9) or their carers (n=8) included preventing deterioration of health, remaining active and independent. Medication related goals of care themes included maintaining adherence, reducing medication burden, and reducing ADRs.
Discussion. Older people have expressed a wide range of health and medication-related goals. Further analysis will determine how an acute hospital setting can influence goals of care compared to a community setting.

407 Exploring consumer's perspective of pharmacist delivering COVID-19 vaccinations

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Introduction. The severe acute respiratory syndrome coronavirus 2 (COVID-19), has significantly impacted the global health system. Efforts to curb the spread of the virus largely rely on mass immunisation. Community pharmacists have played an important role in driving the vaccination efforts in Australia, and as of the 13th of July 2022, administered 7.7 million COVID-19 vaccinations nationwide.
Aims. The aim of this study was to explore the reasons for and attitudes of consumers receiving COVID-19 vaccination from community pharmacists.
Methods. A nation-wide online survey (accessible via QR code) was conducted in Australian community pharmacies between September 2021 to April 2022. To be eligible, participants must be (i) aged ≥18 years and above and (ii) must have received the COVID-19 vaccination at the participating community pharmacies.
Results. A total of 127 individuals participated in the survey and majority (91%) were from South Australia and almost everyone (93%) had received the Moderna® COVID-19 vaccine. Nearly half of respondents (49.6%) had never received vaccinations from community pharmacists before. Commonly chosen reasons for getting vaccinated in community pharmacies were convenience of booking and receiving the vaccine (86%) and easy accessibility of a pharmacy (70%). Whilst most respondents (n=66) did not have any major concerns about receiving vaccination at a community pharmacy, others were concerned about the side effects of the vaccine (n=10), procedure such as pain (n=5), or raised pharmacy specific concerns (n=7). In addition, majority (79%) of respondents agreed or strongly agreed to receive other vaccinations from a pharmacist as well as recommend family and friends to get their vaccinations at a pharmacy (87%).
Discussion. The ease of access was a significant facilitator in consumers opting to get their COVID-19 vaccinations at community pharmacies. Our findings also suggest that the involvement of pharmacists in the national vaccination strategy was positively received by consumers. Future health strategies should utilise the unique skillsets and accessibility of pharmacies and pharmacists.

408 COVID-19 and Mental illness: perceptions of the pandemic and adherence to pandemic public health measures.

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Introduction. Pandemic public health measures (PPHMs) play an important role in controlling the spread of coronavirus disease (COVID-19). Preliminary findings suggest that people with low perception of COVID-19 are more likely to be non-adherent to PPHMs. Literature on patients with severe mental illnesses is limited.
Aims. To assess mental health consumers' knowledge and perceptions of COVID-19 and adherence to PPHMs.
Methods. Face-to-face surveys were conducted at a Community Mental Health Service centre in South Australia (SA) between July to December 2021. The survey included both Likert-scales and open-ended questions. Data collection stopped when the service ceased all non-essential face-to-face contact with patients at a time of increased community restrictions.
Results. Findings from the surveys (n = 22) indicated that majority of the participants (n=16) recognised COVID-19 as a problem in Australia. However, most (n=16) did not believe that COVID-19 had negatively impacted their mental wellbeing. Participants reported 'often' or 'always' wearing masks in public (n=16), whilst more than half (n=12) reported 'often' adhering to social distancing practice. Although most (n=15) report that they were 'likely' or 'very likely' to be tested if they experienced symptoms, only 6 respondents could list at least three COVID-19 symptoms. Additionally, whilst 86% said they would be vaccinated, less than 70% had at least one vaccination by February 2022 (community vaccination rate >90%). Limitations included the small sample size, use of face-to-face surveys (risk of social desirability) and Likert-scales (potentially suggest the 'expected' response).
Discussion. Limited PPHM adherence and awareness of COVID-19 symptoms in this cohort are of concern. As health professionals who play a vital role in patient education, pharmacists can positively contribute to this area. As SA was relatively unaffected by COVID-19 prior to December 2021, replication of this study in other Australian states would be useful. Future studies, with a larger sample size and inclusion of a control group are required as people with severe mental illness often have significant medical comorbidities which may put them at increased risk of poor COVID-19 outcomes, and little is known about their knowledge, attitudes and behaviours related to COVID-19.

409 Development of a survey to evaluate deprescribing guideline dissemination and implementation

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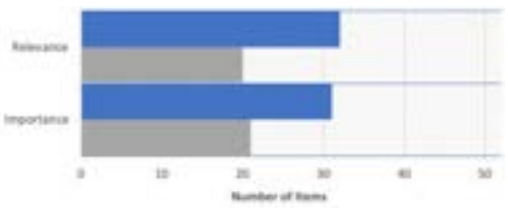
Introduction. Deprescribing guidelines provide evidence-based recommendations to guide clinicians on when and how to reduce and/or cease medications. Previous studies have indicated that policies, protocols, and processes within organisations may facilitate or hinder the adoption of deprescribing guidelines. At present, there is limited knowledge on the strategies used by organisations to disseminate and implement deprescribing guidelines, and their impact.
Methods. An on-line survey was developed to investigate organisations involved in deprescribing guideline endorsement, dissemination, modification, or translation. The survey questions were divided into six sections mirroring components of the Reach, Effectiveness, Adoption, Implementation and Maintenance framework, which address the translation of scientific advances into everyday practice. Stage one involved item generation informed by previous systematic review. Stage two involved content validation established by experts with knowledge and experience in guideline use, development, dissemination, and implementation.
Results. There were 52 survey items developed for the first round of content validation which investigates current dissemination and implementation efforts led by organisations. Thirty-nine out of 52 items (75%) achieved content validity with an I-CVI score of 0.78 or above for relevance and importance. Forty-six items were revised to improve clarity, two were retained, and four were removed.
Discussion. A validated survey may identify effective dissemination and implementation strategies for deprescribing guidelines, gauge their utility and impact, and inform strategies for newly developed guidelines. A second round of content validation is currently underway, following which, the survey will be distributed internationally. Distribution of the survey will uncover the current dissemination strategies and adoption of deprescribing guidelines and accompanying knowledge mobilisation tools.



410 COVID-19's impact on pharmacy innovation and legislative changes

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Introduction. The Australian Journal of Pharmacy (AJP) is a premier pharmacy publication. AJP articles reported legislative and clinical changes throughout the COVID-19 pandemic, and highlighted the extraordinary efforts of Australian pharmacists adapting rapidly to the ever-changing legislative landscape during this time.
Aims. To create a timeline of events as documented by the AJP to track the legislative changes that affected community pharmacy practice and community pharmacy-led innovations in response to these challenges.
Methods. A keyword search was conducted in the AJP for all articles tagged with the terms 'pandemic,' 'COVID-19', 'coronavirus' and 'vaccine' from September 2019 to July 19, 2022. Articles were reviewed to further understand the timeline of pharmacy-related legislative changes and associated innovations.
Results. A total of 665 articles were identified and separated into various subcategories. 263 articles were included in a timeline describing government communications and 123 articles were included in a timeline showcasing pharmacist innovation. The first in a barrage of legislative changes occurred in March 2020 as "panic buying" and distancing restrictions were implemented. Pharmacists adapted existing services to maintain patient safety and accommodate lock-downs; examples include expanded medicines delivery and, later, innovative vaccination outreach programs.
Discussion. Australian pharmacists demonstrated strengths in innovation and adaptability following legislative and social changes. A large number of new strategies have been implemented with the aims to maintain patient safety and improve patient outcomes while maintaining medication provision and professional services. However, there was significant concern about discrepancies amongst states and territories, as not all State Governments chose to implement Federal legislation.

411 Is amoxicillin a suitable antibiotic for home intravenous administration?

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Introduction. Outpatient Parental Antimicrobial Therapy (OPAT) is gaining momentum as an intravenous antibiotic treatment, enabling patients to receive antibiotic treatment in their own homes. Currently there is limited data on the stability of different strengths of amoxicillin and clavulanic acid for parental use in elastomeric infusers.
Aims. To investigate the shelf-life of amoxicillin under kinetic and clinical concentrations, including pH control, and to evaluate its stability in the presence of clavulanic acid for use in OPAT.
Methods. High Pressure Liquid Chromatography was used to evaluate the stability of amoxicillin and clavulanic acid when stored at various pH values (6.5, 7.0 and 8.0), temperatures (40, 45, 52 and 60℃) and concentrations (1.0/0.2, 7.5/1.5 and 15/3.0 mg/mL).
Results. The lowest concentration of amoxicillin and clavulanic acid was most stable at pH 6.5 at 40℃; providing a shelf-life of 4.9 hours for amoxicillin and 1.4 hours for clavulanic acid. When comparing 1 mg/mL and 15 mg/mL amoxicillin at the same temperature and pH range (ie 40°C and pH 7.9 - 8.3), a change in shelf-life of 2.8 hours to 0.1 hours respectively was observed. The kinetics data tests were used to estimate conditions for the elastomeric infuser testing. Amoxicillin 1.0 mg/mL and clavulanic acid 0.2 mg/mL had stability for 165 hours (approximately 7 days); this concentration was found to be the most stable. Studies of 7.5 mg/mL and 15 mg/mL amoxicillin (with clavulanic acid) stored in elastomeric infusers at 2.9°C indicated a shelf-life of amoxicillin of 50.8 hours and 3.1 hours respectively (clavulanic acid approximately 30.1 h and 1.4 h respectively). At the optimum pH for maximum stability (pH 6.5) amoxicillin was insoluble above 5.8 mg/mL[a]. The lowest pH for adequate solubility was pH 8.0.
Discussion. Our study suggests that patients could possibly be administered two 7.5/ 1.5 mg/mL amoxicillin/ clavulanic acid infusers simultaneously over a 24-hour period as this provided greater stability than elastomeric infusers containing 15.0/3.0 mg/mL amoxicillin.

[a] Tsuji A et al. Physicochemical properties of amphoteric beta-lactam antibiotics I: stability and dissolution behaviour of amino penicillins as a function of pH. J Pharm Sci. 1978. 67: 1059-66.

413 Clinical pharmacists' participation in ward rounds in Australian hospitals: The current practice

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Introduction: Pharmacists' participation in ward rounds (WRs) can reduce adverse drug events and improve medication communication. However, there is paucity of data on the current utilization of pharmacists as participants in WRs in Australian hospitals.
Aims: To explore the level of WR participation by clinical pharmacists in Australian hospitals and the enablers and barriers to their participation.
Methods: The 'Theoretical Domains Framework' informed questionnaire for clinical pharmacists in Australia collected demographic information and current WR practices. Responses to key enablers and barriers to WR participation were collected using a Likert-scale. Cross tabulation analysis was conducted to determine the association between the Likert-scale statements and WR participation.
Results: Ninety-nine responses were included in the analysis. Sixty-seven participants had WRs in their respective clinical units. Clinical pharmacists' attendance in WR was low (26/67, 39%) even though 89% of all respondents (88/99) agreed or strongly agreed that participating in WRs is part of their scope of practice. Thirty-nine percent of participants with a WR service attended at least one WR in the preceding fortnight (median of 4 (IQR 2–6) WR in the two-week period). Pharmacists who did not attend WR reported feeling as though there is a lack of awareness of the role of the pharmacist within the WR team compared with those that do attend WR (63% versus 23%, p=0.005) and they do not have enough time to attend the WR (73% versus 31%, p=0.002). Pharmacists who did attend WRs reported that they have support from the inter-professional team to attend the WR compared with those that did not attend WRs (85% versus 37%, p=<0.001).
Discussion: This study highlights the need for ongoing interventions such as re-structuring workflows and increasing the awareness of the role of the clinical pharmacists within a WR to enable continued interprofessional collaboration in practice.

414 What is impacting clinical pharmacists' participation in ward rounds?

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Introduction. Pharmacists' participation in ward rounds (WRs) can reduce adverse drug events and improve medication appropriateness and communication. Available evidence suggests that WR participation by clinical pharmacists is currently limited.
Aims. Using an Australian national survey, we aimed to explore the enablers and barriers to WR participation by clinical pharmacists in Australian hospitals as described by them in free text responses within the survey.
Methods. The survey contained free-text questions relating to enablers and barriers to WR participation such as 'Describe why you do not participate in the ward round in its entirety', and 'I am more likely to participate in the ward round if…'. The free-text questions were analysed thematically in Nivo-2020 according to Braun and Clarke's technique. A reflexive thematic analysis was undertaken.
Results. The five themes constructed from the responses of 99 participants were: 'the clinical pharmacy service structure', 'ward round structure', 'pharmacist's capabilities', 'culture' and 'value'. A culture supportive of pharmacist's contribution to WR's and supported by a consistent WR structure with flexible delivery of clinical pharmacy service enabled pharmacists' participation in WRs. Pharmacists using WR as a platform to undertake clinical pharmacy activities and recognizing the value of being present at the time of decision-making, enabled them to see value in their participation in WR.
Discussion. Being physically 'absent' from the WR due to workload, workflow and self-perception of the need for extensive clinical knowledge can limit opportunities for pharmacists to begin proactive exchanges with the physicians to improve patient care outcomes. Bidirectional communication between the interprofessional team and the pharmacists, where there is mutual understanding and co-construction of each individual's role in the WR facilitates consistent and inter-dependent collaboration for effective medication management.

415 Clinic-based pharmacists: the missing link

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Introduction. "Take at Home Medicines" (TAHM) including antiemetics, antidiarrheals, pre-medications and Granulocyte Colony Stimulating Factor (G-CSF) are crucial in the management of adverse effects of antineoplastic treatments in cancer patients. While a multidisciplinary approach is used for input into patient care plans, pharmacists are not currently involved in the review and patient education of TAHM.
Aims. Determine the incidence of actual and potential medication-related issues associated with TAHM and the impact on patient outcomes and experience.
Methods. A retrospective qualitative review was undertaken in the day chemotherapy unit of an Australian tertiary hospital without a dedicated outpatient oncology pharmacist. Using hospital databases, a random sample of patients receiving new IV chemotherapy between January and December 2021 was selected. The incidence of incorrect prescribing and omission of TAHM were recorded. Reports of adverse drug events in these patients for the first two chemotherapy cycles were also recorded.
Results. Of the 117 records reviewed, medication-related issues including omissions, deviation from accepted protocols and erroneous prescribing were identified in 42 patients (36%). Of these, 12 patients (29%) experienced adverse drug events with 1 patient being hospitalised due to antineoplastic therapy adverse effects. Contributing factors identified included patients not taking TAHM correctly due to misunderstanding of instructions for use (n=2) and TAHM being incorrectly prescribed (n=10).
Discussion. While omission of the patient's antiemetic medication can result in severe nausea and vomiting, omission of a G-CSF dose can increase risk of neutropenia post-chemotherapy. Patients taking chemotherapy supportive medicines for the first time are at risk of medication misadventure due to inexperience, particularly if they have not received adequate education, or if prescribers fail to prescribe the recommended TAHM. The findings of this qualitative study support greater pharmacist oversight to support patients in the understanding of their TAHM.

416 Pharmacist consultations in hospitalised older surgical patients

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Introduction. Older adults are at high risk of medication-related adverse events during hospitalisation for surgery. The improvement of safety and quality use of medicines is a key standard in the National Safety and Quality Health Service Standards. Pharmacists play a key role in the healthcare team to implement this standard.
Aims. To characterise the nature of pharmacist consultations in an older surgical cohort.
Methods. A prospective cohort study of 302 consecutive patients aged ≥65 years admitted to a tertiary vascular surgery unit. As part of routine care, a pharmacist is attached to each ward (rather than by specialty team) and prioritises patient review according to requests from the multidisciplinary team and risk factors for medication-related issues initially identified from daily admission lists, dispensing requests and communication with ward staff. Frequency and type of pharmacist consultations, and associations with patient and clinical characteristics were investigated.
Results. Patients had a median (IQR) age of 79.0 (73.0-84.0) years, 202 (66.9%) were male and 111 (36.8%) were frail (Clinical Frailty Scale score >4). There was a total of 299 pharmacist consultations, with 159 (52.6%) patients being reviewed at least once by the pharmacist. Of the 299 pharmacist consultations, most common interventions were medication order reviews (38.8%) and medication history interviews (21.4%). Pharmacist consultation was more likely if the patient was an emergency admission ($p=0.045$), had an admission to ICU during the hospitalisation ($p<0.001$) or had a long-stay admission defined as > 14 days ($p<0.001$). Older age, frail status, cognitive impairment, presence of polypharmacy and operative management were not associated with having a pharmacist review.
Discussion. In the absence of blanket review of all admitted patients, we found that pharmacist consultations were more likely to occur in older vascular surgery patients who were emergency admissions, had an admission to the intensive care unit or had a hospital stay > 14 days. Patient characteristics such as older age, frailty and cognitive impairment were not associated with pharmacist consultations but represent an important target population for proactive pharmacist consultation.

417 Sleep Health Management in Residential Aged Care Facilities

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Introduction. In humans, sleep is essential for the maintenance of several neurobiological processes such as memory consolidation, mood and metabolic regulation. It is known that sleep patterns vary with age and are affected by multiple factors. While non-pharmacological strategies are generally considered first-line for managing sleep disturbances, sedatives are excessively and inappropriately used in the older population.
Aims. This study aimed to explore the management of sleep health in residential aged care facilities (RACFs) by nurse professionals and to identify the key factors that impact provision of optimal sleep health care.
Method. An inductive thematic qualitative research method was employed to analyse the data collected from semi-structured interviews with registered nurses working in RACFs.
Results. Seventeen interviews were conducted, and the data yielded three themes: 1) the nurses' observations and knowledge of sleep health, 2) the strategies employed in RACFs for the management of sleep disturbances, 3) the organizational barriers to evidence-based sleep health management.
Discussion. Nurse participants reported the use of both non-pharmacological and pharmacological interventions. Sedatives were commonly prescribed due to their fast action and accessibility despite the guidelines indicating their use in later stages. Although benzodiazepines are known for their many side effects, such as drowsiness and oversedation, temazepam was the most commonly administered drug. Sleep in RACFs was affected by several factors such as aging and comorbidities (e.g., dementia, pain, anxiety). However, there were also many modifiable factors that negatively impacted sleep management in RACFs. These include staffing ratios, nursing duties, medication side effects and lack of training and involvement of allied health professionals. This study highlighted the importance of involving a multidisciplinary team and the urge to develop guidelines and training programs for healthcare professionals to improve sleep health management in RACFs.

418 Do entry-level pharmacy students have different personal characteristics?

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Introduction. Schools and faculties of pharmacy are responsible for the education and training of the future generation of pharmacists. Despite many similarities, pharmacists training, and scope for engaging with advanced roles differs between provinces of Canada, as well as with New Zealand.
Aims. To determine the differences in trait characteristics between students entering a New Zealand (NZ) and Canadian pharmacy programme
Methods. All new second year students were invited to take an online questionnaire that included potential predictors of involvement in pharmacists' roles. (1) the Big Five Inventory (openness, conscientiousness, extraversion, agreeableness, neuroticism), (2) the Achievement Goals Questionnaire-Revised, (3) the Rational experiential inventory (4) the Counselor role orientation measure (CRO). Two sample *t*-tests were conducted to determine if there were differences between students from NZ and Canada
Results. 184 students (97 NZ pharmacy, 87 Canada) completed the survey. On average, Canada students had scored higher on extraversion (M=60, p=.15), agreeableness (M=80, p=.06), conscientiousness (M=70, p=0.30), mastery-approach (M=93, p=.06), faith-in-intuition (M=67, p=.03) compared to NZ pharmacy students who were higher for openness M=70, p=.09). The Canada pharmacy students scored high for experiential scale (M=66, p=.03).CRO measures reliance on physician (medicine advice– physician is better, give patients all they need to know") was endorsed more by NZ pharmacy students (M=35, p<.001), than Canada (M=15). Items on time pressure (It takes too much time to for a pharmacist to talk with a patient about the medication they receive) were significantly higher for NZ students (NZ, M=41, p=0.26), Canada (M=37). Higher scores for pharmacist restriction ("There should be legal restrictions on what pharmacists can tell patients") for NZ students (M=26), Canada (M=12, p<.001).
Discussion. Overall, entry-level pharmacy students' had similar personality profiles between Canada and New Zealand. Our future work will determine how these personality and learning goals influence students' preparation for future practice.

419 Predictors of pharmacist's and pharmacy students' involvement in patient-centred pharmacy services

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Introduction. Pharmacists are actively being encouraged to do more patient-centred activities. However, the adoption of patient-centred services into practice is still low, and we do not know which personality characteristics predict the adoption of these services.
Aims. To determine what personality characteristics are associated providing advanced services/roles or intent to do in so the future for pharmacy students and pharmacists.
Methods. NZ pharmacists and final year pharmacy students participated in an online questionnaire containing personality measures including the Big Five Inventory (openness, conscientiousness, extraversion, agreeableness, neuroticism), Achievement Goals Questionnaire-Revised, New General Self-Efficacy and the Counselor Role Orientation measure (pharmacist-restriction, time-pressure, reliance on doctor, side-effect). We used multiple linear regression with the dependent variable being a score for interest in providing advanced pharmacy services and demographic variables and personality measures being the independent variables. A secondary analysis was performed with the dependent variable being a score for interest in traditional pharmacy services.
Results. 268 pharmacists and 83 pharmacy students completed the survey. Pharmacists' intention to perform advanced services was associated by higher scores in extraversion (B=0.30, p<.001), higher agreeableness (B=0.44, p<.001), and higher mastery-approach (B=0.40, p<.001). Pharmacy students intention were associated with higher conscientiousness (0.35,p<.001), higher mastery-avoidance (B=0.16,p<.05), higher self-efficacy (B=0.52, p<.001), higher performance approach (B=0.19,p<.001), higher side effect (B=-0.51,p<.001), higher reliance on doctor (B=-0.49,p<.001),pharmacist restriction (B=-0.34,p<.001), and higher time-pressure(B=-0.37, p<001). Intention to perform traditional services for pharmacists were associated with higher self-efficacy, B=0.43, p<.001), mastery-approach (B=0.36, p<.001) whereas for pharmacy students it was associated with lower academic achievement (B=-1.01, p<.05), and fear of not mastering learning material (mastery-avoidance, B=0.31, p<.05).
Discussion. Understanding characteristics that predict engagement with patient-centred roles will help shape the future of the profession, especially to aid in the workforce planning process.

420 Pharmacy students' role expectations in practice: A comparison between NZ and Canada

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Introduction. Worldwide, the pharmacy profession aims to move towards a patient-centred paradigm. Nationally-endorsed vision documents like the NZ Pharmacy Action Plan 2016-2020 and the Blueprint for Pharmacy (Canada) have outlined a new set of expectations for pharmacists in this role. However, do future pharmacists see themselves as patient-centred?
Aims. To explore predictors of intention to provide advanced and traditional pharmacy roles between entry-level pharmacy students in New Zealand (NZ) and Canada
Methods. All second-year pharmacy students were invited to take an online questionnaire, containing (1) Big Five Inventory (openness, conscientiousness, extraversion, agreeableness, neuroticism) (2) Achievement Goals Questionnaire-Revised and (3) rational versus intuitive decision-making style. A score (0-100) was created for interest in providing new roles (patient centred) and traditional roles (drug distribution and checking). Descriptive and comparative analyses were conducted to evaluate the characteristics and responses to the questionnaires. Univariate linear regression was used to determine whether each scale was associated with the outcome measure.
Results. 184 students (97 NZ pharmacy, 87 Canada) completed the survey. The graph depicts the difference between intentions to do pharmacy roles between students. Intention to perform new roles was predicted by higher conscientiousness, (B=0.43, p<.002) and a higher need for cognition (B=0.35, p<.005) among NZ students vs. higher mastery-approach (B=0.19, p<.05) for Canadian students. Intention to perform traditional roles was predicted by higher agreeableness (B=0.28, p<.001, need for cognition (B=0.28, p<.001) among NZ students, and higher mastery-approach (B=0.23, p<.001, performance-avoidance (B=0.10, p<.001) among Canada students.
Discussion. These findings highlight the need for educators to take into account the influence of traits on the intention to adopt patient centred roles. Follow up with these cohorts will determine whether these are predictive of future role engagement, as well as the influence of workplace experiences.

421 Optimising adherence to allopurinol and gout management: people with gout's perspectives

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Introduction. Despite safe, affordable and effective urate-lowering therapy such as allopurinol, gout management is suboptimal. Poor adherence to allopurinol contributes to suboptimal gout management, with adherence being reported lower than other chronic conditions that require prophylactic medication.
Aims. To understand the facilitators and barriers of adherence to allopurinol across the stages of medication adherence, including opinions of strategies to improve adherence.
Methods. Semi-structured interviews were conducted with people with gout (N = 26), previously or currently taking allopurinol. De-identified verbatim transcripts were thematically analysed by two researchers independently.
Results. Participants reported facilitators of adherence during allopurinol initiation were motivation to prevent gout flares and trust in their healthcare professionals' (HCP) advice. Reluctance to commence long-term medication was a barrier to initiation. Believing in the effectiveness and necessity of allopurinol and using reminder systems were facilitators of implementation. Barriers to implementation included forgetfulness, experiencing gout flares, and receiving limited feedback on allopurinol's effectiveness. Participants discontinued therapy when allopurinol was perceived as ineffective or unnecessary, they experienced gout flares despite adhering, or received suboptimal advice on gout. Participants identified receiving accurate advice by HCPs and regular urate results for feedback on allopurinol's effectiveness as potential strategies to improve adherence. Perceived benefits of self-monitoring urate to promote adherence included the ability to self-manage gout and make informed decisions about their gout with their HCP.
Discussion. People with gout's perceptions of the effectiveness and necessity of allopurinol influences intentional adherence during initiation, implementation and discontinuation. Strategies that inform people with gout of their urate control, respond to their experiences with gout, and provide accurate medical advice have the potential to improve gout management including adherence to allopurinol.

422 20 years of obstetric medicines information calls – a timeline of enquiry patterns and trends

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Introduction**.** An Obstetric Medicines Information Service has been collating data since establishment in 1988, with electronic documentation of enquiries since 2001. Despite comprising 20 years of call records, this database had not been analysed to determine whether the service meets callers' requirements and compares to similar services.
Aims**.** To evaluate the database of medicine information enquiries over a 20-year period (2001-2020).
Methods**.** Records of enquiries from 2001 to 2020 were screened, coded and analysed to identify demographic data and trends over 20 years. Descriptive, bivariate and multivariate analyses were guided by research questions relating to medicines use in breastfeeding and pregnancy identified from the literature.
Results**.** Following data cleaning, 48,458 enquiries were analysed, with 48.2% (n=23,334) pertaining to breastfeeding and 42.1% (n=20,425) pertaining to pregnancy. Health consumers were the predominant users of the service, but declined from 60% of callers in 2001 to 38% in 2020. Enquiries relating to medicines use in breastfeeding (48%, n=23,334) outnumbered those relating to pregnancy (42%, n=20.425). The most common period of concern was the first trimester (n=6,201, 36.2%). Of the 23,334 calls that related to breastfeeding, 16,905 (72.4%) recorded the age of the infant, with the majority (24.3%) within the first four weeks of an infant's life. Antimicrobials dominated the enquiries, representing 19.5% of calls (n=9,454), followed by antidepressants, analgesics and complementary medicines. 373 enquiries (0.8%) related to use of recreational substances in pregnancy or breastfeeding. Five COVID-19-related enquiries were recorded in the database.
Discussion. Evaluation of the database provided an understanding of patterns of medicines use and areas for education, training and development to assist the users of the service. The predominance of enquiries relating to the vulnerable first trimester of pregnancy highlights the value of this service. Increase in health professionals' use of the service over 20 years reflects changes in the complexity of medication and prescribing considerations and supports the ongoing need for this specialised service. The richness of these data warrant ongoing investment in the service.

423 Investigating medicinal cannabis as a treatment for spasticity and muscle spasms

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Introduction. Appropriate treatment of spasticity and muscle spasms is important as these conditions have the potential to significantly impair patients' quality of life. However, conventional pharmacological treatments for spasticity and muscle spasm have a number of limitations including poor efficacy and/or tolerability. As such, newer therapies such as medicinal cannabis are being explored as a potential treatment option.
Aims. This study aimed to investigate the effectiveness and adverse events of numerous medicinal cannabis products for treating spasticity and muscle spasms which have been caused by various underlying conditions.
Methods. An interim analysis of the CA Clinics Observational Study (CACOS) was performed, where patients were provided with surveys regarding patient demographics, medicinal cannabis usage, health-related quality of life (HRQoL) outcomes, and adverse events. Patients were eligible for inclusion in our study if they experienced spasticity or muscle spasms. Data from the surveys was used to conduct a range of statistical analyses to determine the most effective ratios of Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) for patients, the most effective routes of administration (i.e. oral or inhaled), and the most common adverse events experienced by patients.
Results. From more than 2000 patients enrolled in CACOS, 150 were eligible for enrolment in this study. This included 72 patients (48%) who reported spasticity and 78 patients (52%) who reported muscle spasms. The mean age of the patients was 50 (SD = 13.57). The three most common underlying conditions causing spasticity and muscle spasms in this data set were multiple sclerosis, spinal injuries, and fibromyalgia. A total of 69 patients (46%) were cannabis naïve before commencing medicinal cannabis treatment. There were 74 patients (49%) still using medicinal cannabis but 15 patients (10%) ceased treatment due to reasons such as cost and intolerable adverse events. A further 61 patients (41%) were not followed up, switched doctors, or died. Data analysis on effectiveness and rates of adverse events remains ongoing.
Discussion. Medicinal cannabis is proposed to alleviate muscle spasms and spasticity by reducing the skeletal muscle hyperexcitability associated with these conditions; however, our preliminary results suggest that factors such as cost and adverse events may pose as potential barriers to treatment.

424 Are Essential Medicines Accessible?

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Introduction. Inadequate medication access is a pressing concern throughout the world. To gain insight into this issue, World Health Organisation (WHO) and Health Action International (HAI) constructed a validated methodology for surveying medicine prices, availability, and affordability.
Aims. In 2008, a publication from The Lancet (Cameron et al., 2009) synthesised relevant WHO/HAI data from 36 countries, revealing inadequate medication access. Here we present an updated systematic review and meta-analyses of available WHO/HAI data.
Methods. The HAI Essential Medicines Access Database and four electronic databases were searched, identifying 105 studies that employed the WHO/HAI methodology. A total of 86 studies were included, spanning 64 individual countries across the world. Data concerned with availability, affordability and pricing were extracted and synthesised. A meta-analysis was performed on 15 core medicines found throughout most studies.
Results. Average public sector availability of generic medicines ranged from 13.1% to 72.5% and 14.8% to 82.5% within low-income and low-middle income countries, respectively. Patients within the private sector paid, on average, 7.9 times international reference prices for generic medicines. Treatments for acute and chronic diseases remained unaffordable in many countries, often costing several days' wages to purchase a course of medication.
Discussion. Medication within the public and private sectors of low-middle income countries remains unavailable and unaffordable. The implications of this are yet to be explored. Policy modifications such as promoting generic medication or alternative procurement methods are required to improve affordability and increase availability.

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425 A scoping review of health-system guidelines for opioid dispensing.

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Introduction. Inappropriate use of prescription opioids, particularly when used to treat chronic non-cancer pain, contribute significantly to upward-trending statistics of opioid-related harms. While the introduction of programs such as Real-Time Prescription Monitoring has equipped health-practitioners with more information to make clinical decisions, an argument is made that the use of these programs increases the level of responsibility of dispensing pharmacists. Appropriate guidance alongside these programs is required to support these pharmacists in clinical decision making.
Aims. To evaluate the current health-system guidance available to pharmacists dispensing opioids and to examine the implications of this guidance on pharmacist responsibility.
Methods. We conducted a scoping review searching in CINAHL, MEDLINE, Embase, PubMed and Web of Science, in addition to the grey literature and referral from topic experts to collate a list of current health-system guidelines relevant to pharmacists dispensing opioids. These guidelines were then examined through a narrative review and the use of the "Appraisal of Guidelines Research & Evaluation—Health Systems" tool (AGREE-HS).
Results. Ten guidelines met the inclusion criteria and were analysed in the study. We found that while health-systems guidelines often contain some level of practical advice for pharmacists, the advice given lacked consistency between guidelines, revealing the lack of consensus in this area. Many of the guidelines examined failed to provide evidence of systematic and evidence-based development techniques. Overall, the guidelines included in this study lacked tangible advice that supported the responsibilities of pharmacists dispensing opioids.
Discussion. The current health-system guidance on opioid dispensing is inadequate, and demonstrates the need for a high-level ethical framework to support pharmacists dispensing opioids. This scoping review of health-systems guidelines not only provides an argument for further investigation, but offers direction in the key considerations for future framework development.

426 The codesign and evaluation of an osteoporosis module by teachers and pharmacists

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Introduction. Osteoporosis is a paediatric disease with geriatric consequences. Fostering healthy bone behaviours during adolescence may reduce the incidence of, and disastrous outcomes of, poor bone health in older age.
Aims. To develop and evaluate bone health educational materials for NSW PDHPE students from years 7-10.
Methods. A codesign approach was used to develop the modules, involving semi-structured stakeholder meetings with endocrinologists, academic pharmacists, PDHPE teachers, and students. The modules were implemented in 9 NSW high schools. A pre-post quiz was conducted to evaluate knowledge change. Interviews were conducted with students and teachers to guide widespread implementation in the NSW high school curriculum. Thematic analysis was conducted using the Theory of Planned Behaviour.
Results. The codesign process resulted in 4 modules which were rated as highly acceptable to teachers and students. Average knowledge scores significantly improved from 81.25% at baseline to 87.50% ($p<0.001$) in tests taken immediately post module delivery. Interviews after module delivery revealed high levels of satisfaction among students and teachers. Students expressed increased awareness of importance of bone health "*I realised that I need to be doing a little bit better and taking care of my bones in a more serious way".* Students indicated that they intended to undertake some preventive health behaviours, such as obtaining calcium in the diet, but seemed only somewhat willing to regularly do weight-bearing exercise.
Discussion. A collaborative approach has resulted in highly engaging modules for high school students that improves knowledge and may result in healthy behaviours to be adopted to improve bone health.

427 The importance of medication information to community dwelling individuals

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Introduction. Providing people with medication information improves the correct and safe use of medications. It is the responsibility of health professionals to provide accurate, medication information in a manner that is tailored to suit the individual. Assumptions made by health professionals about what people want to know about their medications may take away their empowerment and sense of shared responsibility for their medication management.
Aims. To identify the importance of Consumer Medicines Information (CMI) medication topics for community dwelling individuals.
Methods. This cross-sectional study utilized an online questionnaire to survey community dwelling individuals. Respondents scored the importance of the 13 CMI medication information topics on a 5-point Likert scale, from Not at all important (0) to Extremely important (EI, 4). The responses were summed to provide a total importance score (TIS, maximum score of 52)
Results. Some useable data was obtained from 301 people, with 249 complete responses. Respondents were aged between 18 and 88, 84% were female, with 41% holding a Bachelors degree or higher, 6% were Aboriginal or Torres Straight Islanders, 4% were born outside of Australia, 12% were Aphra accredited health professionals and 80% were taking at least 1 regular medication. The percentage of respondents reporting that a CMI topic was EI varied from 12% (Name of the inactive ingredient/s) to 83% (Dosage of the medicine). The TIS ranged from 22 to 52 (*Md*=44 *IQR*=40 to 48) and 5%(16) respondents indicated all 13 topics were EI. There was no difference in the TIS for Health professionals (*Md*=44, *n*=38) and non-health professionals (*Md*=45, *n*=192). The TIS for males respondents (*Md*=42, *n*=33) was lower than for female respondents (*Md*=45, *n*=197), *U*=4397, *z*=3.25, *p*=0.001, *r*=0.21. Pharmacists (64%) were the most frequently selected source of medication information, along with doctors (57%) and respondents reported less frequent use of the internet (34%) and CMIs (33%).
Discussion. As frequently used sources of medication information pharmacists and doctors need to provide information at the level desired by their patients. The importance of medication information to community dwelling individuals varies widely and male patients are likely to consider medication information less important than female patients.

428 Pharmacists' acceptability of perinatal depression screening in community pharmacies: A content analysis

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Introduction. Perinatal depression (PND) screening helps the timely identification and referral of individuals at high risk of PND. Screening can be delivered by different primary healthcare professionals including pharmacists.
Aim. To explore pharmacists' views and acceptability of PND screening in community pharmacies.
Methods. A questionnaire, including 42 Likert-scale type questions, demographic questions and three-open ended questions exploring acceptability, training and resource needs, was distributed to pharmacists in Australia through professional organisations and social media. This study specifically focuses on the content analysis conducted on responses to the three open-ended questions. Inductive content analysis was used to identify categories and sub-categories. Sub-categories were then deductively mapped to the theoretical framework of acceptability (TFA) (constructs: Affective attitude, Burden, Perceived Effectiveness, Ethicality, Intervention Coherence, Opportunity Costs and Self-efficacy)[1]
Results. Four major categories emerged from 149 responses: *benefits of PND-screening, accessibility of community pharmacists and pharmacies, physical environment* and *system and policy changes.* Fourteen sub-categories were deductively mapped to the TFA. The potential benefits of community pharmacist-led PND screening was highlighted, whereby accessibility was a facilitator and lack of privacy in pharmacies was a barrier. Furthermore, respondents questioned whether such services were within pharmacists' scope of practice. Pharmacists felt that further training and additional resources (e.g., promotional material, reimbursement) were needed for the delivery of community pharmacist-led PND screening.
Discussion. Overall, pharmacists acknowledged the benefits of PND screening service; however, indicated the need to adapt training and resources specifically for this purpose. The physical environment of the pharmacy and staffing were also essential to consider. Findings may guide the implementation and evaluation of pharmacist-led PND screening.

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429 Prevalence of fluoropyrimidine-induced toxicities in patients receiving fluoropyrimidine-based chemotherapy

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Introduction. In Australia, fluoropyrimidines (FP) are amongst the most frequently prescribed chemotherapy agents. FP can cause severe toxicities in up to 40% of patients leading to early disruption of treatment, underdosing and even death. Treating FP-induced toxicities is very costly - average costs for the treatment of FP-induced severe diarrhoea and moderate anaemia are AUD$4,821 and AUD$17,100 respectively and treatment for neutropenia can be up to AUD$12,054.
Aims. To study the prevalence of FP toxicities in patients receiving FP-based treatment at a tertiary Australian hospital.
Methods. A retrospective clinical audit of all patients over 18 years of age who received their first dose of fluorouracil or capecitabine at the Gold Coast University Hospital between 1st January and 31st December 2020 was conducted. The electronic medical record of each patient was analysed for known FP toxicities and details of adverse events (AEs) were recorded, including severity according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0). Subsequent management including changes or delays to the chemotherapy regimen were also investigated.
Results. A total of 215 patients were included in this study: 107 patients received fluorouracil and 108 patients received capecitabine. A total of 1083 AEs were reported. The most frequently reported toxicities in order of decreasing prevalence were fatigue (233 AEs; 21.5%), diarrhoea (164 AEs; 15.1%) nausea (157 AEs; 14.5%), hand and foot syndrome (89 AEs; 8.2%), mucositis (78 AEs; 7.2%) and neutropenia (44 AEs; 4%). The most severe toxicities (grades 3 or 4) were neutropenia (34 AEs; 3.1%), diarrhoea (23 AEs; 2.1%), nausea (8 AEs; 0.7%) and chest pain (3 AEs; 0.3%). Treatment delays occurred in 62 instances, dose reduction occurred in 47 instances and cessation of therapy occurred in 47 instances.

Discussion. In Australia, toxicities from FP are common and can be of extreme severity. Adverse events may result in treatment delays and early cessation of therapy, potentially impacting clinical efficacy and quality of life. Further research is planned to investigate the relationship between DPD deficiencies and FP toxicities.

430 Barriers and enablers to the use of parenteral methotrexate: a scoping review.

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Introduction. Methotrexate (MTX) is the first-line treatment for rheumatoid arthritis (RA) and is effective in controlling disease activity. Parenteral MTX may have benefits over oral MTX, but it is currently used infrequently in clinical practice. It is necessary to explore the patient perspective of the use of parenteral MTX therapy.
Aims. To describe the barriers and enablers to the use of parenteral MTX from the perspective of patients with RA and to identify the research gaps in this field.
Methods. A scoping review was conducted via a search of Medline, Embase, Scopus and the Cochrane Library from inception to May 31, 2021. Data synthesis was conducted using the Theoretical Framework of Acceptability. Any type of study, which was conducted in adults, explored the use of parenteral MTX in RA from the patients' perspective and was written in English was included in the review.
Results. Fifteen studies were included; findings related to the constructs "affective attitude", "burden", "intervention coherence" and "self-efficacy" were explored the most while some were rarely ("opportunity cost" and "perceived effectiveness") or not ("ethicality") investigated. RA patients were generally satisfied with MTX injections ("affective attitude"). Ease of using a self-injection device, limited pain, high perceived efficacy, a sense of independence when self-administering and general positive perceptions were identified as enablers to parenteral MTX use. Limited dexterity to handle the injection device (leading to difficulties removing the cap etc), concern about side effects, loss of autonomy if not able to self-administer and negative perception about MTX were barriers.
Discussion. Multiple barriers and enablers to the use of MTX injections have been described in the literature. Further qualitative research is required to explore the barriers and enablers to the use of MTX injection in depth, as most of the included studies were quantitative in nature and focused on specific brands of MTX prefilled pens.

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Starr R et al (2005) Pharmacology of FAB-4, ed Ono Y. pp 12-23, Tokyo, Abbey Road Press

431 Availability and evaluation of medication management resources for carers of people with dementia: a scoping review with an environmental scan

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Introduction. Carers are vital in managing medications for people with dementia but have indicated that guidance may be inadequate, and they often seek out web-based and physical resources. However, the availability and scope of resources to guide informal carers of people with dementia on medication management has not been explored.
Aims. To identify and evaluate what resources are available for carers of people with dementia that provide guidance in medication management.
Methods. A systematic search of MEDLINE, Embase, CINAHL and PsycINFO was performed in May 2022 to identify and evaluate resources for carers of people with dementia that provide guidance in medication management. Google and known repositories of dementia were also searched. The readability of text-based resources was examined through the Flesch-Kincaid reading level, the Flesch reading ease and the Gunning-Fog index. Resources were further evaluated using the Patient Education Material Assessment Tool (PEMAT) to gauge resource understandability and actionability. The protocol for this study has been registered on OSF, registration DOI: 10.17605/OSF.IO/GV8HF
Results. 5217 records were screened, and 15 medication management resources were identified by two independent reviewers. Resources most commonly included guidance on medication administration (n=14), and strategies on how to manage refusal of medications (n=11). Existing resources did not appear to demonstrate a co-development approach involving people with dementia or their carers or address high-risk care settings. Readability scores required an average level of Grade 10, and only two text-based resources were satisfactory in meeting the average reading level. PEMAT scores had an average of 84% for understandability and 71% for actionability.
Discussion. There are limited resources that provide both practical and holistic guidance in the medication management process. There is a clear need to co-develop accessible and understandable resources that provide medication management guidance for carers across settings to address the multi-faceted nature of dementia care.

432 Application of user-centred co-design principles to address barriers in therapeutic drug monitoring

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Introduction. Different software applications have been developed to support healthcare professionals with individualised drug dosing. However, poor usability and integration into workflow have partly limited their uptake in practice. This likely represents the limited involvement of healthcare professionals in the development of drug dosing software. This study applied co-design principles using the experiences and expertise of healthcare professionals to inform the design of dosing software to address barriers in therapeutic drug monitoring (TDM), using vancomycin as an example.
Aims. To identify pharmacist and prescriber barriers in TDM, and to explore how drug dosing software can address these barriers.
Methods. A series of three workshops were conducted with pharmacists and prescribers. User journey storyboards, personas and prototyping tools were used to explore existing barriers to practice and opportunities for innovation through drug dosing software design. A prototype of a software interface was presented to participants for feedback.
Results. 11 hospital pharmacists and 6 prescribers with ≥2 years of TDM experience were recruited. Participants identified a lack of confidence in vancomycin dosing and pharmacokinetic understanding, and difficulty in accessing practice guidelines as key barriers which could be addressed through software implementation. Accessibility to information (e.g. guidelines and pharmacokinetic resources) and ways to visualise and communicate data depended on the needs and experience of the user.
Discussion. Drug dosing software need to be adaptable to the needs and workflow of clinical users. The whole clinical context of the patient also needs to be considered. The involvement of healthcare professionals in the development of clinical tools, as well as training and education, is needed to promote tool utilisation and improvement in practice.

433 Exploring health literacy responsiveness within remote pharmacy organisations

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Introduction. Health literacy describes an individual's ability to access, understand, appraise, remember and use health information to make appropriate health decisions. Pharmacies are often the first access point for healthcare information and therefore play a crucial role in health literacy development for individuals and the community. This is particularly important in remote Australia due to the disproportionate health literacy disparity that exist, compared to metropolitan areas. By applying the Organisational Health Literacy Responsiveness (Org-HLR) Tool, pharmacy staff can identify initiatives to improve healthcare that will benefit patients with any level of health literacy.
Aims. To explore the experiences and perceptions of remote pharmacy staff using the Org-HLR Tool, and to identify potential barriers and enablers for implementing health literacy responsive initiatives within remote pharmacies.
Methods. A qualitative study was conducted amongst pharmacy staff in a remote region of Western Australia. After engaging in a series of workshops which delivered the Org-HLR Tool, participants took part in semi-structured interviews to discuss their experiences. Interviews were audio recorded, transcribed verbatim, coded and analysed via the Framework method.
Results. The eight pharmacy staff found the Org-HLR Tool helpful for improving health literacy responsiveness within their pharmacies. They reflected on the presence of staff, health literacy understanding, overall experience of the workshops and workshop structure. They identified that lack of time and funding along with organisational constraints could provide barriers to implementation, while community engagement, health literacy knowledge and support from management and other organisations could enable implementation.
Discussion. Overall, remote pharmacy staff felt that the Org-HLR Tool was beneficial to their pharmacy practice, but identified barriers that could delay or prevent implementation. By focusing on achievable initiatives, remote pharmacies can improve their health literacy responsiveness and, in turn, improve the health literacy of the community.

434 Are we sleeping on melatonin? An exploration of Australian pharmacists' perspectives and attitudes towards the down-scheduling of melatonin.

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Introduction: Insomnia is a common complaint in community pharmacy. With the down-scheduling of PR-Melatonin, the repertoire of non-prescription sleep aids has expanded, further complicating pharmacists' clinical decision-making process. However, little is known about how pharmacists have responded to this regulatory change, or its impact on pharmacists' scope of practice.
Objectives: This study aims to explore how the down-scheduling of melatonin after June 2021, has impacted Australian community pharmacists, and their perceived preparedness towards responding to the regulatory change.
Methods: A mixed-methods study was conducted in a convenience sample of community pharmacists and pharmacy interns. An online questionnaire captured participants' knowledge and attitudes toward melatonin. Semi-structured interviews were conducted to gain insight into current practice on the provision of melatonin. Interviews were digitally recorded, transcribed verbatim and analysed using the Framework Approach to identify emergent themes.
Results: 72 surveys were returned (interns, n=23; pharmacists, n=49) and 17 interviews were conducted (interns, n=4; pharmacists, n=13). From the survey, work processes remained the same for 60% of participants since the down-scheduling with no notable changes in the uptake of other sleep aids. Three key themes emerged from the preliminary analysis of the interviews: 1) Business as usual, 2) Reliance on patient honesty, and 3) Knowledge gaps and uncertainties. Interns and pharmacists welcomed the availability of melatonin in community pharmacies. However, the age restriction imposed (i.e., ≥ 55 years) placed pharmacists in a difficult position of needing to educate younger patients while gatekeeping the appropriate and safe use of melatonin.
Conclusion: Results allude to a generally positive consensus towards the recent down-scheduling of PR-Melatonin. However, pharmacists and intern pharmacists highlighted the need for more education and training resources, specifically safety and efficacy data, to support their practice beyond the procedural focus of supplying melatonin.

435 Determination of physical compatibility of oxycodone and co-administered drugs via intra-venous ports

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Introduction. Multiple drug administration through the intravenous (IV) route in hospitals is necessary due to the critical state of hospitalized patients who need multi-drug therapy with a limited number of IV lines accessible. This risks the occurrence of physical incompatibilities between concomitant drugs leading to line blockages, jeopardizing patient safety and therapy efficacy. Error rates were reported in multiple drug IV administration in hospital wards which were mainly attributed to a lack of or limited data on the compatibility of the drugs. Oxycodone is frequently prescribed for pain management in acute care settings, however, sufficient data on associated combinations is currently lacking.
Aims. Performing physical compatibility analysis of oxycodone combinations injected via Y-site infusion and providing reliable data to implement in hospital units with minimal health risks.
Methods. The limited data on oxycodone combinations was identified by anaesthetists and intensivists. Accordingly, the selected drugs included Ketamine, Clonidine, Tramadol, Vancomycin, Dexmedetomidine, Piperacillin-Tazobactam, Gentamicin, and Cefotaxime. Physical compatibility assays were confirmed based on the absence of visual & subvisual precipitation, turbidity, color change, gas evolution, and pH changes. Drugs were mixed in the ratio of 1:1 in conditions simulating hospital settings (room temperature and unprotected from light) and physical compatibility was determined at time points 0 and 60 minutes after mixing.
Results. The tested combinations showed physical compatibility at the maximum prescribed doses over 60 minutes.
Discussion. The proposed investigations led to the generation of critical information which is currently lacking and could contribute to evidence-based decisions and facilitate the optimization of oxycodone-containing treatment protocols.

436 Psychotropic adverse drug event monitoring tools for residential care: a systematic review

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Introduction. International guidelines recommend psychotropic adverse drug event (ADE) monitoring for people living with dementia and in residential aged care facilities (RACFs). There are no published comparisons of psychotropic ADE monitoring tools for this purpose.
Aims. To evaluate properties and characteristics of psychotropic ADE monitoring tools intended for use in RACFs.
Methods. Medline, CINAHL, Embase and PsycInfo databases were searched from inception to August 2022 for studies that reported the development, validation, or use of psychotropic ADE monitoring tools. Data extraction and quality assessment was performed independently by two investigators. Quality was assessed against the following criteria: test-retest reliability, inter-rater reliability, content validity and construct validity.
Results. From 1834 identified articles, eight articles describing six psychotropic ADE monitoring tools were included. The tools monitored antipsychotic (n=6 tools), benzodiazepine (n=4 tools) and antidepressant (n=4 tools) ADEs. Four tools were designed for use by nurses, one by general practitioners, one by multidisciplinary teams. Two tools reported it took 10-60 minutes to assess potential ADEs. Five tools described steps to take if a suspected ADE was detected. Outcomes from the use of the tool include changes in psychotropic prescribing. Resident feedback about the application of one tool was reported. Test-retest reliability and construct validity was not reported adequately for any tools. Inter-rater reliability was reported for two tools. Content validity for three tools was established through development by a multidisciplinary team.
Discussion. Psychotropic ADE monitoring tools were primarily designed for use by nursing staff. ADEs were escalated to other members of the multidisciplinary team, with involvement of residents or their relatives/proxies reported infrequently. The complexity of existing tools may preclude their use in routine clinical practice. A psychotropic ADE monitoring tool suitable for RACFs should feasibly integrate into routine clinical care and facilitate multidisciplinary optimization of medication safety. Further research using rigorous scientific methodology is required to assess the applicability of existing and future psychotropic ADE monitoring tools.

437 Prevalence and predictors of long-term opioid use following orthopaedic surgery in Australia

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Introduction. Opioid analgesics prescribed for the management of acute pain following orthopaedic surgery may lead to unintended long-term opioid use and associated patient harms.
Aims. This study aimed to examine the prevalence of opioid use at 90 days after elective orthopaedic surgery across major city, regional and rural locations in New South Wales, Australia.
Methods. We conducted a prospective, observational cohort study of patients receiving elective orthopaedic surgery at five hospitals from major city, regional, rural, public and private settings between April 2017 and February 2020. Data were collected by patient questionnaire at pre-admission clinic held two to six weeks before surgery and telephone call after 90 days following surgery.
Results. Of the 361 participants recruited, 54% (195/361) were female and mean age was 67.7 (standard deviation 10.1) years. Opioid use at 90 or more days after orthopaedic surgery was reported by 15.8% (57/361; 95% confidence interval [CI], 12.2% – 20%) of all participants and ranged from 3.5% (2/57) at a major city location to 37.8% (14/37) at an inner regional location. Predictors for long-term postoperative opioid use in the multivariable analysis were surgery performed at an inner regional location (adjusted odds ratio [aOR], 12.26; 95% CI, 2.2 – 68.24) and outer regional location (aOR, 5.46; 95% CI, 1.09 – 27.50) after adjusting for known covariates.
Discussion. Long-term opioid use was reported in over 15% of patients following orthopaedic surgery and appears to be more prevalent in regional locations in Australia.

438 Understanding pharmacists' scope of practice in managing asthma in the community

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Introduction. There is evidence that the management of asthma is suboptimal in the community (Azzi et al, 2019; Azzi et al, 2022). Pharmacists are in an ideal position to manage asthma in the pharmacy; however, they might not be well equipped or face challenges in daily practice.
Aim. To investigate pharmacists' perception of their role with regard to asthma management.
Methods. This study took the form of a self-completed electronic survey (based on empirical evidence and asthma management principles). A convenient sample of pharmacists was recruited from the community. The survey explored pharmacists' perceptions of pharmacy management, barriers and needs. Data were analysed descriptively.
Results. Among 128 pharmacists who completed the survey, 75% work in a metropolitan area; 58% reported working in a service-oriented community pharmacy. Under ideal circumstances, 91% of pharmacists reported that their role included checking inhaler technique, 91% reported assessment of symptom control and 89% reported adherence. In day-to-day practice, 85% reported their role included checking inhaler technique, 84% checking reliever medication use and 82% explored symptom control. The most common barriers reported were lack of time (82%); patient disinterest (51%) and remuneration (48%). A maximum of 62% of pharmacists reported unmet educational needs. The highest areas of need related to education around lung function testing (spirometry and peak expiratory flow), comorbidities and impact on asthma, action plans and knowledge around asthma flare-ups.
Discussion. There appear to be some differences between pharmacists' perception of their role under ideal circumstances and the reality of delivering care in day-to-day practice. Medication management with regard to inhaler technique and reliever overuse were a priority, as was symptom control. A lower-than-expected proportion of pharmacists reported education needs in some areas, especially those areas in which they were not delivering care. The barrier of time, remuneration and patient disinterest in receiving asthma care remain. A deeper exploration of pharmacists' perceptions may be needed to uncover novel solutions.
[1]Azzi et al (2019) BMJ Open 9(8): e028995. [2]Azzi et al (2022) J Asthma 59(1):178-188.

439 The impact of medicinal cannabis legalisation and re-scheduling on poisonings in Australia

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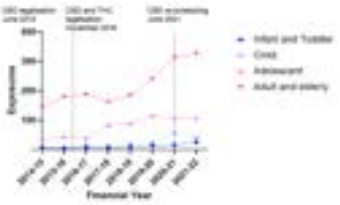
Introduction. The Therapeutic Goods Administration (TGA) has recently re-scheduled cannabis and tetrahydrocannabinols due to the increase in demand for medicinal cannabis. Re-scheduling alters access to cannabis, which could potentially have an impact on harm, including poisonings.



Aims. To evaluate the effect of medicinal cannabis legalisation and re-scheduling on poisonings.
Methods. Time-series analysis of calls regarding cannabis exposures to Australia's largest Poisons Information Centre (PIC), July 2014-June 2022. Joinpoint regression analysis was used to examine whether there were any significant changes in trend (changepoints) and calculate the average annual percent change (AAPC) in exposure calls.
Results. There were 2630 poisoning exposures to the NSW PIC over the study period. Of these cases, 82% (n=2149) were intentional exposures, and 18% (n=481) were unintentional. All age categories noted a rise in exposure calls (Figure). Most (66%, n=1735) were adults (20 years and over), followed by adolescents (15-19 years, 24%, n=619), children (5-14 years, 7%, n=178), and infants and toddlers (<5 years, 3%, n=87) (figure). Joinpoint analysis showed a significant increase in cannabis exposures, with an AAPC of 15.3% (95% CI: 12 to 18%, $P < 0.001$), 2014-15 to 2021-22, with no changepoints detected.
Discussion. There was a significant increase in cannabis exposures in the period after the legalisation. It is important that public health agencies consider applying harm minimisation approaches to limit the impact of cannabis legislation on acute poisonings, especially as legalisation of recreational cannabis continues to be debated.

440 Paracetamol (acetaminophen) hepatotoxicity increases in the presence of an added herbal compound

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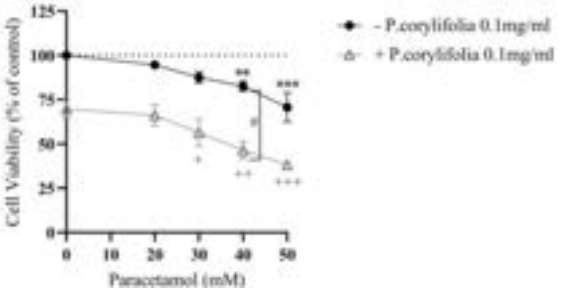
Introduction. Hepatotoxicity from paracetamol/acetaminophen has occasionally been reported at lower-than-expected doses. Often attributed to idiosyncratic response, the possibility of unpredicted interactions with complementary herbal medicines may provide further clarity to these responses considering the heightened popularity of herbal products.



Aims. As herbal preparations may interact with pharmaceutical drugs the following in vitro study was undertaken to determine whether the toxic effects of paracetamol on liver cell growth in culture would be exacerbated by the addition of the furanocoumarin compounds present in Psoralea corylifolia, a common Chinese herb.
Methods. The study utilised liver carcinoma cell line (HepG2) cultures to assess hepatotoxicity in the presence of increasing concentrations of paracetamol (0–50 mM) and P. corylifolia (0–5 mg/ml) alone and in combination. MTT colorimetric assays demonstrated cell viability after 48 h.
Results. P. corylifolia was significantly toxic from 0.3 mg/ml to 5 mg/ml ($p < 0.05$), whereas paracetamol was not toxic below 50 mM ($p = 0.0026$). Interactions between previously non-toxic levels of 0.1 mg/ml of P. corylifolia and increasing concentrations of paracetamol (0–50 mM) were observed, with a significant increase in toxicity compared to paracetamol alone (30% cell death vs. 72% cell death with P. corylifolia). A significant synergistic interaction was observed at 40 mM paracetamol with 0.1 mg/ml of P. corylifolia ($p = 0.038$).
Discussion. This study has shown significantly increased hepatotoxicity in cell cultures exposed to paracetamol when herbal compounds containing furanocoumarins were added. Fulminant acute liver failure occurring after the ingestion of low doses of paracetamol may not, therefore, always be due to an occult idiosyncratic response to paracetamol, but instead possibly to the combined effects of paracetamol and herbal preparations.

441 Could herbal soup be a potentially unrecognised cause of hepatotoxicity at autopsy?

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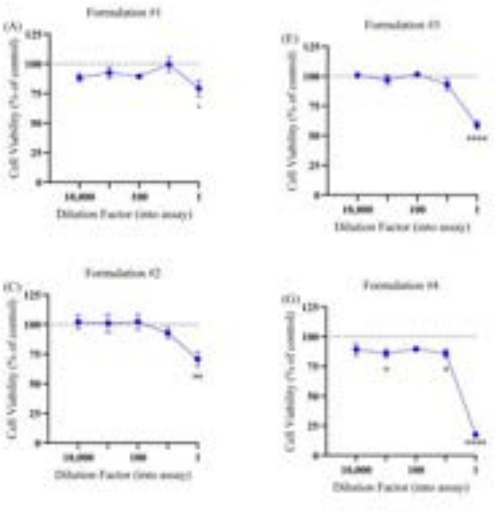
Introduction. Unexpected hepatic failure with liver necrosis is sometimes encountered during a forensic autopsy. Determining the aetiology may sometimes be difficult, although increasingly herbal medicines are being implicated.
Aims. To determine whether such effects might also be cause by foodstuffs containing herbal products, the following in vitro study was undertaken.
Methods. Four formulations of traditional herbal soup advertised as *bak kut teh* were prepared and added to cultures of liver carcinoma cells (HepG2). Cell viability was assessed using MTT colorimetric assay at 48h.
Results. All formulations have significant toxicity prior to dilution ($p < 0.05$). Formulation #1 showed 21% cell death ($p = 0.023$), formulation #2 30% ($p = 0.009$), and formulation #3 41% ($p < 0.0001$). Formulations #1-3 showed no significant toxicity once diluted ($p > 0.05$). Formulation #4 showed approximately 83% cell death before dilution ($p < 0.0001$) and persistent toxicity even with dilutions at 1:10 (15% ± 3.7, $p = 0.023$) and 1:1000 (14% ± 3.8, $p = 0.024$).



Discussion. This study has shown that herbal foodstuffs such as *bak kut teh* may be responsible for variable degrees of in vitro hepatotoxicity, thus extending the range of herbal products that may be potentially injurious to the liver. If unexpected liver damage is encountered at autopsy, information on possible recent ingestion of herbal food preparations should be sought as routine toxicology screening will not identify the active components. Liver damage may therefore be caused not only by herbal medicines but possibly by herbal products contained in food.

442 The impact of pharmacist only up-scheduling of modified release paracetamol on poisonings in Australia

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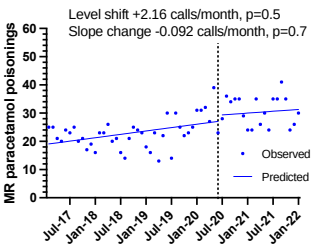
Introduction. In response to rising rates of overdose with modified release (MR) paracetamol, Australia's Therapeutic Goods Administration (TGA) up-scheduled MR paracetamol from Schedule 2 to Schedule 3 in June 2020.
Aims. To evaluate the impact of MR paracetamol up-scheduling on poisonings.



Methods. An interrupted time series analysis of calls to the NSW Poisons Information Centre. Exposure calls between February 2017 and January 2022 relating to MR paracetamol were included. Immediate release (IR) paracetamol, ibuprofen and other over-the-counter (OTC) analgesics were used as a comparison.

Results. There were 22,950 intentional exposures to OTC analgesics: MR paracetamol (n=1522), immediate release paracetamol (n=12,540), paracetamol/ibuprofen combination (n=297), ibuprofen (n=6071), diclofenac (n=536), mefenamic acid (n=226), naproxen (n=497), and aspirin (n=1261). MR paracetamol exposes continued on an upward trajectory following the scheduling change (figure), with an average of 23 exposures/month pre-intervention and 31 exposures/month post-rescheduling. There was no significant level shift or slope change for MR paracetamol (level shift +2.16 calls, p=0.5, slope change -0.092 calls/month, p=0.7). IR paracetamol had a significant level shift (+53.7 calls, p<0.001), as did ibuprofen (level shift +22.5 calls, p<0.001), possibly indicating some switching behaviour. However, the proportion of OTC analgesic calls regarding MR paracetamol was unchanged (6.8% vs 6.5%, p=0.23).

Discussion. The increase in poisonings with MR paracetamol does not appear to have been attenuated by re-scheduling in the 20 months following the intervention. MR paracetamol is more toxic in overdose than IR paracetamol, and further up-scheduling to Schedule 4 could be considered.

443 Biochemical changes in liver and intestinal cells induced by weight loss supplements

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Introduction. The rise in obesity has been associated with increased demand for weight loss supplements (WLS). Although popular, these products are regulated via a 'trust-based system' which does not require them to be independently analysed prior to approval, despite a lack of research into the safety of WLS as complex chemical mixtures. In recent years, adverse events associated with WLS have risen, with liver damage being a common concern. Prior studies into the safety of WLS predominantly focused on individual ingredients or active compounds, therefore knowledge of their toxicity as mixtures containing many potentially active compounds is lacking. For this reason, understanding of the biochemical changes in response to WLS is limited.
Aims. The purpose of this study was to examine whether WLS reduce liver and colon cell viability and, if so, determine the biochemical changes associated with this toxicity.
Methods. The toxicity of 10 herbal weight loss supplements was investigated using HepG2 and Caco-2 cells. Cells were exposed to 0.1, 0.3, 1.0 and 3.0 mg/mL of each weight loss supplement for 48 h, then assessed for cytotoxicity using the MTT assay. WLS found to induce significant toxicity and reduced cell viability by 25-50% were selected for further analysis. Here, biochemical changes associated with toxicity were determined using ^{1}H-NMR spectroscopy.
Results. Nine WLS induced significant cytotoxicity in HepG2 cells, with six causing a decrease in cell viability of 25-50%. All 10 weight loss supplements were observed to induce cytotoxicity in Caco-2 cells, and eight reduced cell viability by 25-50%. A total of 14 supplement/cell line pairings were selected for ^{1}H-NMR analysis, with biochemical changes relating to oxidative stress occurring in response to the treatments.
Discussion. WLS have been increasingly associated with cases of liver damage, and this research demonstrates that this may occur via mechanisms of oxidative stress. Changes in metabolites involved in oxidative phosphorylation suggest mitochondrial dysfunction as a key mechanism of toxicity. Intestinal health is also of concern, with oxidative stress also observed in Caco-2 cells. This multicellular toxicity coupled with the poor regulation of these products indicates that WLS may pose a serious risk to consumers, despite the popular belief that these substances safe.

444 QSAR models to detect endocrine-disrupting chemicals acting at the retinoic acid receptor

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Introduction. The retinoid signalling pathway is currently being advocated for inclusion in OECD endocrine disruptor testing guidelines due to its key role in the hormonal regulation of growth, development, and reproduction, as well as crosstalk with other endocrine pathways. Accordingly, there is regulatory demand for quantitative structure – activity relationship (QSAR) predictive models that enable the screening of retinoid-based endocrine disrupting chemicals.
Aims. To develop QSAR models predicting activity at the retinoic acid receptor (RAR), the main nuclear receptor of the retinoid pathway, and to compare the effect of different types of molecular descriptors on the accuracy and interpretability of these QSAR models.
Methods. A dataset of approximately 7,000 chemicals tested in a high-throughput RAR assay was extracted from the U.S. Tox21 program database. Ligand-based descriptors were calculated with the Mordred v1.2.0 library and QSAR models developed using the random forest classifier from the scikit-learn v1.0.2 library.
Results. Preliminary models developed with traditional one- and two-dimensional ligand-based descriptors perform at approximately 60% balanced accuracy. Additional models are being developed with more mechanistic three-dimensional descriptors that also encode structural information about the RAR binding site and are expected to predict with higher accuracy.
Discussion. Computational prediction of receptor-based molecular initiating events may be hindered by traditional usage of QSAR descriptors that encode information about the ligand only. Greater consideration of the structural features of the binding site that surrounds the ligand is required to derive more realistic descriptors that can improve the predictivity and interpretability of toxicological QSAR models.

445 Bisphenols depress contractile activity of gut smooth muscle in rats

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Introduction. Bisphenols are chemicals widely used in the plastic industries for manufacture of various plastic items including water bottles, baby feeding bottles and other food and beverage containers. These toxic chemicals are known to leach to food and beverage items and can be detected in body fluids. The ill effects of bisphenols on endocrine system and metabolism as well as their oestrogen like actions are well documented. However, their effects on gut smooth muscle are less studied, despite the fact that the primary route of entry of these chemicals is via gut.
Aims. The present study was aimed to assess gut smooth muscle contractile activity on acute exposure to bisphenols in adult and neonate rats.
Methods. The experiments were carried out after obtaining the approval from institutional ethical committee for animal experiments. Gut segments from small and large gut were prepared from overnight fasted male albino rats (adult and neonate) and isometric contractile activities *in vitro* were recorded with the help of a standard organ bath and digital recording systems (ADI, Australia) at different bath concentration (1-100 µM) of bisphenols. In addition, various antagonists (atropin, L-NAME, tamoxifen, and hexamethonium) were used to evaluate the mechanisms of action. Parameters studied were contractile tension and frequency.
Results. The results showed that bisphenols (1-100 µM) significantly ($p<0.05$, two way ANOVA, *t*-tests) depress contractile tension and frequency of various gut segments in a dose dependent manner. Further, atropine, L-NAME, tamoxifen failed to block bisphenol-induced attenuation in contractile responses.
Discussion. The present study clearly demonstrated that bisphenols reduce the contractility of gut smooth muscles. The mechanisms of depressive action of bisphenols could not be ascertained, however, appeared to be independent of oestrogen receptors, nitric oxide and cholinergic system. The present investigation suggested that bisphenols used in plastic food containers may alter the gut motility under *in vitro* conditions and may be a potential contributor to the development of gastrointestinal motility disorders.

446 Nitromethane toxicokinetics estimated from a case study

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Introduction. Nitromethane interferes with the serum creatinine Jaffe reaction, however its disposition after overdose is not well known (Baselt 2020).
Aim. To describe the toxicokinetics of nitromethane after ingestion.
Methods. Case study of a 14-year-old patient (47 kg) who drank a liquid containing 70% methanol and 20% nitromethane. Jaffe creatinine concentrations of plasma spiked with nitromethane were determined on the same analyser. Assumptions about nitromethane: MW 61 g/mol; 1.122 g/mL at 35˚C; F 100%; plasma concentration stable during initial 4 h. Assumptions about methanol: MW 32 g/mol; 0.7734 g/mL at 36˚C; F 100%, peak plasma concentration reached within 30 min and stable for 4 h; Vd 0.7 L/kg; zero-order elimination of 2.6 mmol/L per h (Graw et al 2000).
Results. 4 h after overdose, serum creatinine (Jaffe) was 458 µmol/L (enzymatic 39 µmol/L). Spiking experiment: Jaffe creatinine (µmol/L) = endogenous content + 0.531 nitromethane (µmol/L). Therefore, nitromethane concentration at 4 h was 790 µmol/L; its t1/2 was determined as 13.4 h (804 min). Methanol: 6 h after overdose, serum concentration was 4.4 mmol/L. Cmax was 9.6 mmol/L; amount ingested would have been 316 mmol (10.1 g, 13.1 mL). Consequently, the ingested amount of nitromethane would have been 3.7 mL (4.2 g, 69 mmol); its Vd was estimated as 87 L (1.89 L/kg) and CL as 108 mL/min.
Discussion. This is the first description of a complete toxicokinetic profile after nitromethane ingestion. It may help in the future management of victims poisoned with nitromethane on its own or in combination with methanol.

Baselt RC (2020) Disposition of toxic drugs and chemicals in man, 12th ed. p 1507, California, Biomedical Publications
Graw M et al (2000), Invasion and distribution of methanol, Arch Toxicol 74, p 313-321

447 Plastic released toxic chemical delays the gastric emptying time: Experimental evidence

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Introduction. It is well known that plastic containers used for food and beverages may release toxic chemicals like Bisphenol-A (BPA) to contaminate the edibles. BPA has been reported to adversely affect various functions of the body including reproduction, metabolism and development. However, the immediate or chronic effect of BPA ingestion on gastric motility is poorly understood. Aims. The present study was undertaken to examine the effects of oral BPA exposure on gastric motility and gastric emptying time by determining the gastric transit time of food bolus in adult male albino rats. Methods. To understand the immediate effects, the animals (rats of Charles Foster strain weighing 150–200 g) were fed with one time food pellets containing BPA (2 µg/kg and 50 µg/kg) and in chronic experiments, the animals were provided food pellets containing BPA (50 µg/kg/day) daily for 28 days. Rats fed with same amount of food without BPA served as control. Subsequently, transit indices like gastric transit time was determined by standard charcoal marker method. The animal experiments were performed only after the approval by the Institutional Animal Ethical Clearance Committee. Results. A significant ($p<0.05$) increase in gastric emptying time with nearly 20% increase in gastric transit time/index was observed with different doses of BPA ingestion. Discussion.The present experiments demonstrated that BPA like plastic toxin delays the gastric emptying time.

Tuleu C, (1999) Int J Pharm 180:123-31
Richter CA,*et al* (2007) Reprod Toxicol 24: 199-224

448 Poisonings in older people with dementia: a systematic scoping review

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Introduction. Older people with dementia are increasingly prescribed medications to manage concurrent chronic illnesses. However, they are more predisposed to medication errors and poisoning than people without dementia due to cognitive impairment. Despite this high risk, few studies have investigated and described the nature, prevalence and outcomes of poisonings in this population.
Aims. The objectives of this review are to (i) determine the key agents associated with poisonings in people with dementia, (ii) describe the nature, outcomes and prevalence of these poisonings and (iii) identify and quantify the risk factors that lead to their occurrence.
Methods. A systematic scoping review was conducted using the Joanna Briggs methodology to map this area of international literature. Medline, Embase, PsychInfo and CINAHL databases were searched for publications between September 2001 to September 2021 that reported on poisonings in people with diagnosed dementia. Data charting of the identified aims was conducted, followed by a thematic and numerical descriptive analysis of the findings.
Results. This review's initial search yielded 4,579 eligible articles, of which 18 primary studies were included within the final analysis. Results will be discussed in full, yet preliminary data shows that the therapeutic agents which were most associated with poisonings were Nervous System agents. Unintentional poisonings were described more than intentional poisonings, and clinical care and patient-related risk factors were most frequently identified as risk factors for exposures. Most articles retrospectively investigated poisonings that led to hospitalisations in comparison to other outcomes such as mortality or referral.
Discussion. Nervous system medications, particularly psychoanaleptics, are a significant contributor to therapeutic poisonings in older adults with dementia. Non-therapeutic substances including soap and hand sanitiser contributed greatly to poisonings. Further studies are required to address strategies to safely manage substances in older people with dementia and implement uniform poisoning classification systems and definitions to improve comparability across studies.

449 Biochanin A and quercetin modulate simvastatin toxicity in HepG2 cultures

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Introduction. Phytochemicals such as quercetin and biochanin A found in herbal medicines have evidence of inhibiting cytochrome (CYP450) metabolism and organic anion transporting polypeptide (OATP). These pathways are important to the uptake and metabolism of statins such as simvastatin which is one of the most prescribed drugs worldwide to treat hypercholesteraemia and inhibition of these pathways can lead to statin adverse events.
Aims. To establish if quercetin and biochanin A modulate simvastatin toxicity at comparable concentrations to the drugs diltiazem, and gemfibrozil known to inhibit uptake and metabolism of simvastatin.
Methods. Firstly, the presence of CYP450 and OATP1B1 were validated on our HepG2 cell models. CYP450 activity was confirmed inducing paracetamol's CYP450 dependent toxicity by pre-treating the cells with rifampicin. OATP1B1 activity was measured using the fluorescent probe pyranine. Individual cytotoxicity of simvastatin, quercetin and biochanin A was determined as well as the individual toxicity of the positive controls, gemfibrozil, and diltiazem. Gemfibrozil is a specific OATP inhibitor and diltiazem is a specific CYP3A4 inhibitor. Simvastatin will be co-administered with both positive controls as well as quercetin and biochanin A to assess toxicity modulation.
Results. Paracetamol produced concentration dependent toxicity, and this was induced with the pre-treatment of rifampicin. There was significant, concentration-dependent uptake of pyranine. Simvastatin also reduced cell viability in a concentration dependent manner.
Discussion. The preliminary data confirms the presence of CYP450 and OATP1B1 in HepG2 cultures and that an observable and significant reduction in cell viability is produced by simvastatin. Further experiments can now be conducted investigating the individual cytotoxicity of diltiazem, gemfibrozil, biochanin A and quercetin and subsequently co-administration of these compounds with simvastatin.

Turner RM et al (2019). J Clin Med 9: 22
Yang W et al (2017). Xenobiotica 47: 86-92.

450 Machine learning exploration of per and poly fluoroalkyl substance (PFAS) toxicity

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Introduction. PFAS (per-fluro-alkyl-substances) are ubiquitous. They have been detected in penguin eggs in Antarctica and polar bears in the Arctic which shows how penetrant and persistent they are. The toxicology of most PFAS molecules remains poorly understood. Although some PFAS are no longer in use it is estimated that 600 molecular species are currently in use in the USA (Fenton, 2021).
Aims. The Aim of this project is to construct a benchmark database of PFAS molecules with bioassay results from public chemical safety databases and employ cheminfomatic techniques to generate predictive models of biological assay outcomes to enable risks to be ranked and future studies to be prioritised.
Methods. Python based cheminformatic methods were used to generate chemical databases and build models. Molecular data was gleaned from the Tox21 database with results from seven separate assays. PFAS molecules were then extracted using SMARTS based substructure pattern matching resulting in 1301 valid molecules with the inclusive "Chem.MolFromSmiles('C(F)(F)')" pattern. Machine learning approaches were used to build QSAR models linking physicochemical properties to bioassay outcomes.
Results. Of the assays and activities investigated to date agonist activity at ERRs (estrogen related receptors) and hPXR (human pregnane X receptors) both showed signs of strong relationships within the database with preliminary AUCROC values of 0.66 and 0.72 for ERRs and hPXR, respectively.
Discussion. Although the complete toxicological picture for PFAS molecules remains unclear these preliminary results are supported by similar findings in animal models in the literature (Fenton, 2021). Further models and analysis will be presented including groupings with maximum common substructure (rdMCS) and fingerprint similarity algorithms. Grouping and classifying PFAS molecules on the basis of chemical similarity and assay prediction performance rankings will potentially contribute to guidance of control measures for these persistent environmental chemicals.
Fenton S, et al., (2021) Env Tox Chem 40(3):606-630

451 Tumour Microenvironmental Features Direct Stem Cells Fate

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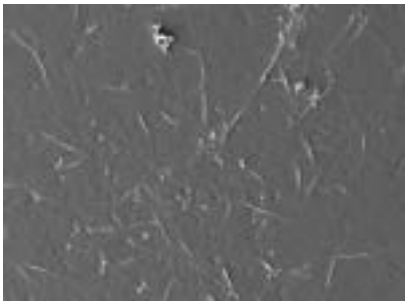

Introduction. The tumour comprising a heterogeneous microenvironment can influence tumour cell behaviour as well as different resident and recruited stroma cells. Mesenchymal stem cells are residing in different cancers and influence cancer cells and tumour microenvironment (TME).
Mesenchymal stem cells shape a part of the tumour stroma and have been reported to have a cancer supporting roles through different mechanisms[1]. The TME is an integral factor of malignancies in different cancers. In this study, we captured the TME breast cancer topographical features by imprinting method to test the effect of the TME on the MSCs.
Methods. To imprint the TME topographical features, the MDA-MB-231, and MDA-MB-468 cell lines (metastatic breast cells) was cultured on 6 well plates. After reaching the desired confluency (>90%), they were fixed by 4% glutaraldehyde. The polydimethylsiloxane (PDMS sylgard 184) polymer with the 10:1 w/w monomer to cross linker ratio was cast on the fixed cells to engineer the tumour cell membrane features as well as the TME topographical cues. The adipose derived MSCs were seeded on top of engineered substrate for 21 days.
Results. The TME topographical cues can enhance the integrin beta-3 gene expression and downregulate E-cadherin. Furthermore, the results of immunohistochemistry staining confirmed that it can enhance the protein expression of αSMA as the biomarker of myofibroblast cells and ki67 as the marker of cell proliferation[2]. Together, the tumour micro and nano topographical cues direct mesenchymal stroma cells to acquire tumour cell-like characteristics.

1. Shi, Y.; Du, L.; Lin, L.; Wang, Y. Tumour-associated mesenchymal stem/stromal cells: emerging therapeutic targets. *Nature reviews Drug discovery* 2017, *16*, 35-52.
2. Rudnick, J.A.; Kuperwasser, C. Stromal biomarkers in breast cancer development and progression. *Clinical & experimental metastasis* 2012, *29*, 663-672.

452 Fragment screening against stabilised α_{1A}- and α_{1B}-adrenoceptors identifies novel sub-type selective antagonist.

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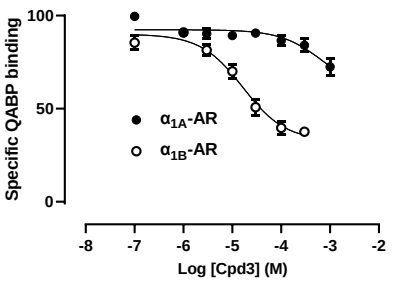

α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (α_1-ARs) are members of the adrenoceptor G protein-coupled receptor (GPCR) family activated endogenously by adrenaline and noradrenaline. They are clinically targeted by non-subtype-selective antagonists, such prazosin and tamsulosin, for the treatment of hypertension and benign prostatic hyperplasia. Their abundant expression in the heart and CNS places them as potential clinical targets for the treatment of various cardiovascular and CNS disorders such as heart failure and Alzheimer's disease. However, understanding their physiological roles and involvement in disease has been hindered by the lack of subtype-selective tool compounds, especially for α_{1B}-AR. Our generation of solubilised, ultra-stable α_{1A}-AR and α_{1B}-AR has allowed application of a biophysical fragment-based drug discovery screen. The screen identified a novel, selective α_{1B}-AR antagonist (Cpd3) that was validated on WT receptors. Computational and site-directed mutagenesis studies identified Cpd 3's binding pocket and provided insight into the molecular basis of α_{1B}-AR subtype selectivity. Optimisation of Cpd3 into a higher affinity ligand will provide a useful laboratory tool or a clinical lead compound, which is needed to probe the physiological roles of specific α_1-AR subtypes and examine their potential as targets for treating disease.

453 Assessment of glucose-lowering constituents in *Teucrium Polium* extract

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Introduction. The prevalence of type 2 diabetes mellitus is rising globally. Although several treatments are available for type 2 diabetes mellitus, more medications are needed to assist with the optimal management of the different stages of the disease. *Teucrium polium* L. (*Tp*) is a herb that has a folk reputation for its antidiabetic potential. Previous studies indicate that *Tp* extracts significantly ($p<0.05$) decrease blood glucose levels *in vivo* and induce insulin secretion from pancreatic β-cells *in vitro*. Although, the constituent/s responsible for this action have not yet been elucidated, select flavonoids (namely, apigenin and quercetin) that are also constituents of this plant have been shown to have glucose lowering potential when tested individually *in vitro* and *in vivo*. Aims. To examine the insulin secretagogue potential of apigenin and quercetin, and their human glucuronide metabolites. Methods. Insulin secretagogue effect of *Tp* extract (from 250 µg of arial parts) and selected flavonoids/metabolites were evaluated using pancreatic beta cell lines INS-1 and BRIN-BD11. Insulin release was determined with the aid of the Insulin ELISA kits, and Glucose uptake assays. Results. Insulin release and glucose uptake increased in response to *Tp* extract in 5.5 mM glucose compared to glucose alone. Apigenin and Quercetin at 100 µM, increased insulin secretion; however, insulin release was more evident with Apigenin-7-glucuronide and Quercetin-3-glucuronide and their combination was more effective. Discussion. In our current study, *Tp* extract promoted insulin secretion from pancreatic beta cells as previously seen by us and others with further compelling evidence that select flavonoid constituents within the extract are responsible for this insulin secretion. However, insulin secretion in the presence of Apigenin-7-glucuronide and quercetin-3-glucuronide exceeds that of the aglycones and this was even more evident when tested in combination, suggesting a possible synergistic effect. These compounds may well provide the basis for a new insulin secretagogue but more research to examine their toxicity and synergistic effect is needed.

454 Validation of positive allosteric modulators for the delta opioid receptors to treat gastrointestinal motility disorders

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Introduction. Opioid medications are highly effective pain relievers or anti-diarrheals. However, the use of opioid drugs is limited by unwanted side effects including respiratory depression, dependence and opioid-induced bowel dysfunctions. Recently, there has been an increasing interest in targeting allosteric sites of opioid receptors in order to reduce both off- and on-target side effects, in particular, positive allosteric modulators (PAMs) of the delta opioid receptor (DOR). BMS-986187 was recently reported as a selective DOR-PAM.
Aims. Investigating novel DOR-PAMs in their ability to modulate enkephalins and how this modulation is affected depending on the signalling pathway engaged.
Methods. In recombinant Chinese Hamster Ovary (CHO) cells stably expressing the human DOR, we characterised and validated the allosteric properties of 50 analogues of BMS-986187 in modulating Leu-enkephalin in two different functional assays, extracellular signal-regulated kinases (ERK) 1/2 phosphorylation, and cAMP inhibition.
Results. We have identified three groups of analogues based on their cooperativity ratios: i) BMS-986187-like analogues ($\alpha\beta_{pERK}>\alpha\beta_{cAMP}$), MIPS3430-like analogues which display equipotent cooperativity ($\alpha\beta_{pERK}=\alpha\beta_{cAMP}$), and MIPS3714-like analogues, which display reversal cooperativity ratios ($\alpha\beta_{pERK}<\alpha\beta_{cAMP}$). Preliminary ex vivo study with MIPS3430 and MIPS3614 in mice colon muscle strip contraction assays showed stronger modulation of Leu-Enkephalin activity by inhibiting neurogenic contractions.
Discussion. To date, our knowledge on which signalling pathways drive which physiological outcomes remains largely unknown for the DOR. With chemical tools displaying distinct cooperativity ratios, we can, for the first, start to link *in vitro* activity in specific signalling pathways and *in vivo* efficacy, in particular, in the context of gastrointestinal disorders.

455 Gs priming of non-Gs protein activation and signalling at the glucagon-likepeptide-1 receptor

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Introduction. The glucagon-like peptide-1 receptor (GLP-1R) is a class B1 G protein-coupled receptor (GPCR) and a very important therapeutic target for the treatment of type-2 diabetes and obesity. The GLP-1R preferentially couples Gs when activated by endogenous peptide agonists, leading to the production of cAMP, however the GLP-1R also couples to other G protein subtypes including Gq, which results in the release of intracellular calcium. However, it remains unclear how the GLP-1R can pleiotropically couple to more than one class of G protein, and the contribution of individual transducers to integrated cellular responses.
Aims. This study aimed to explore the contribution of Gs and Gq to downstream signalling and to gain mechanistic insight into how the receptor can couple to both classes of G protein in response to peptide and non-peptide ligands.
Methods. cAMP production and calcium mobilisation were measured in response to increasing concentrations of ligand in wild-type (WT) and CRISPR-Cas9 HEK cells lacking either endogenous Gs (ΔGs) or Gq/11(ΔGq/11) proteins. The effect of Gs on heterotrimeric Gq protein activation was monitored in HEK ΔGs cells using TRUPATH. All cell lines stably expressed the GLP-1R at similar levels. The agonists assessed include GLP-1 (the endogenous ligand), Peptide-19 (dual incretin receptor peptide), exendin-4 (4FDA approved GLP-1R agonist) and PF 06882961 (small molecule ligand).
Results. All agonists promoted cAMP production in HEK WT and HEK ΔGq/11 cells, but responses were abolished in HEK ΔGs cells. Furthermore, while Gq/11 was essential for calcium mobilisation, with responses abolished in HEK ΔGq/11 cells, calcium responses were also heavily impaired in HEK ΔGs cells. Restoration of Gs expression in HEK ΔGs cells rescued GLP-1R mediated calcium response. Further experiments revealed Gs overexpression increased the dissociation of the Gq or G11 heterotrimer (using TRUPATH) in both HEK ΔGs cells and cells lacking all G proteins.
Discussion. Our data corroborate known findings implicating Gs and Gq as the main G proteins that mediate cAMP production and calcium signalling, respectively, downstream of GLP-1R activation. Our data also reveals that Gs is required for GLP-1R calcium signalling that results from the ability of Gs to enhance GLP-1R-mediated activation of Gq and G11 proteins. We therefore propose that Gs primes the GLP-1R into its active conformation for subsequent coupling and activation of Gq/11.

456 Using cryo-EM to solve the P2X1 receptor structure – a target for male contraception

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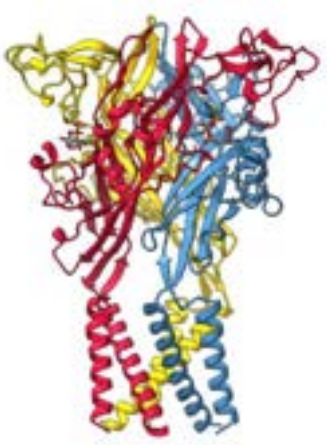
Introduction. 331,000 unintended pregnancies are conceived every day yet, there are only two male contraceptives available. A novel target for male contraception is the P2X1 receptor which is genetically validated but further studies are held back by a lack potent P2X1 receptor antagonists.
Aims. To accelerate drug discovery efforts at the P2X1 receptor this project aims to solve a high-resolution structure of the P2X1 receptor and use this information to guide the design of highly potent P2X1 receptor antagonists.
Methods. Full-length human P2X1 receptor was purified using a membrane purification preparation. Cryogenic transmission electron microscopy (cryo-EM) was used to solve the P2X1 receptor structure. P2X1 receptor antagonists were validated using a HEK293 P2X1 expressing cell line in an intracellular calcium mobilisation assay and a radioligand binding assay.
Results. Initial cryo-EM images of the P2X1 receptor revealed severe preferred orientation of the receptor in vitreous ice. The addition of a secondary detergent, fluorinated FOS-Choline-8, significantly reduced preferred orientation which assisted in obtaining a 1.96 Å structure of the P2X1 receptor in an ATP bound state. The activity of P2X1 receptor antagonists were validated in pharmacology assays.

1.96 Å ATP-Bound P2X1 Model



Discussion. The next step is to generate novel and more effective P2X1 receptor antagonists leveraging our high-resolution P2X1 receptor structure and optimised cryo-EM workflow.

457 Molecular dynamics simulations reveal a novel cholesterol binding site for P2X7 receptors

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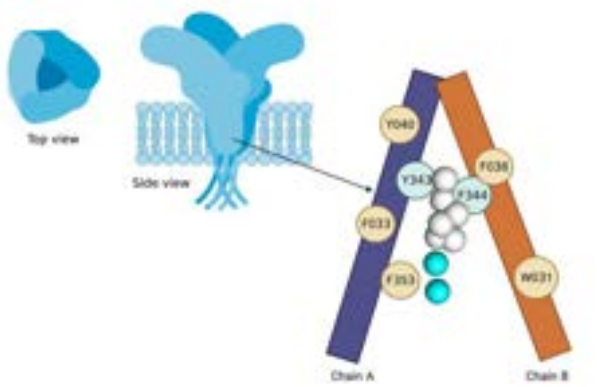
Introduction. P2X7 receptors are trimeric ligand gated ion channels that can be hyper-activated to form a "pore" conformation, leading to cell death. This is associated with poor patient prognosis in inflammatory conditions such as COVID-19. Cholesterol has been demonstrated to be inhibitory to P2X7 action, however its mechanism of action remains poorly understood.

Aims. Using coarse grained molecular dynamics simulations of P2X7 receptors in an epithelial membrane model to determine potential cholesterol binding sites.

Methods. We performed coarse grained molecular dynamics (MARTINI) simulations in GROMACS, then analysed protein-lipid contacts to determine residues and binding sites of interest.

Results. Simulation results reveals a previously uncharacterised cholesterol binding site in the P2X7 receptor transmembrane domain. Furthermore, palmitoylation of the P2X7 receptor is demonstrated to be essential to cholesterol binding.



Discussion. Identification of the cholesterol binding site is key for understanding P2X7 receptor regulation. These cholesterol binding sites could be targeted for finding and optimising novel anti-inflammatory compounds.

Robinson et al (2014) Plasma Membrane Cholesterol as a Regulator of Human and Rodent P2X7 Receptor Activation and Sensitisation. J Bio Chem Vol. 289, 46, pp 31983-31994.

458 The orexin receptors exhibit spatiotemporal signalling mechanisms in response to their endogenous ligands.

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Introduction. The orexin system is a neuromodulatory system comprised of two G protein-coupled receptors (GPCRs), orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2) along with two endogenous ligands cleaved from the same precursor, orexin A (OxA) and orexin B (OxB).

Aims. In regard to orexin receptor signalling, it is most commonly communicated that OxR1 signals through $G\alpha_q$ while OxR2 signals through $G\alpha_q$ and $G\alpha_i$. However, this does not accurately portray the complexities that have been described for the orexin system. The aim of this project was to profile orexin receptor pharmacology within the current framework of GPCR pharmacology.

Methods. Cutting-edge Bioluminescence Resonance Energy Transfer (BRET) techniques were employed to conduct an in-depth investigation of the pharmacology of the orexin receptors with their endogenous ligands

Results. Pharmacologically relevant complexities in the spatio-temporal control of orexin receptor signalling following stimulation with OxA and OxB were observed. In particular, treatment with each ligand resulted in divergences in kinetic trends for G protein activation.

Discussion. These novel findings highlight previously unknown complexities and ligand specificities within the orexin system. This warrants further investigation to determine the relevance of these spatio-temporal trends to different cell types and small molecule orexin agonists.

459 Computational modelling of heterotrimeric P2X7 receptors

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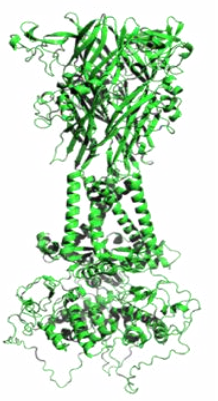

Introduction. P2X7 receptors are trimeric ATP-gated ion channels that have been suggested to participate in the pathophysiology of inflammatory, neurodegenerative and proliferative diseases. Alternative splicing has resulted in the production of P2X7 receptor splice variants (named P2X7A to P2X7L) that can associate to form heterotrimeric P2X7 receptors. The formation of these heterotrimeric receptors complicates the assignment of receptor function as well as drug development.
Aims. This study aimed to investigate the structure of heterotrimeric P2X7 receptors using computational methods, specifically studying the P2X7A/A/L and P2X7A/L/L receptors.
Methods. The artificial intelligence based computational modelling software, ColabFold, on NCI Gadi was used to develop models of the human P2X7A/A/A (wild-type receptor), P2X7A/A/L and P2X7A/L/L receptors. The generated models were validated via molecular dynamics simulations using GROMACS with CHARMM36 force field parameters.
Results. The wild-type, P2X7A/A/L and P2X7A/L/L receptor computational models were generated. The overall structure of the receptor was consistent between the predicted models, however there were significant differences at the ATP binding interface when comparing the wild-type with the heterotrimeric receptor models.
Discussion. These ground-breaking models offer the first insight into the structure of P2X7 receptor splice variants. This is critical for understanding the function of P2X7 receptors in disease states. Our discovery enables further research into these previously unknown conformations.

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Skarratt et al (2020) A P2RX7 single nucleotide polymorphism haplotype promotes exon 7 and 8 skipping and disrupts receptor function. The FASEB Journal 34(3):3884-3901

460 Measuring intracellular calcium signalling dynamics between cell-in-cell structures using genetically-encoded calcium indicators

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Introduction. Cell-in-cell (CIC) structures, where one cell is completely enclosed within another, have long been observed under physiological and pathological conditions (Bauchwitz, 1981; Mackay et al, 2019). One pathological state where CIC structures are observed includes breast cancer, particularly in fluid exudates taken from clinical samples from patients with more advanced and more aggressive cancers (Mackay et al, 2019, Fais et al, 2018). Calcium (Ca^{2+}) signalling plays a crucial role in many cellular processes and alterations in Ca^{2+} signalling are well-established as playing a role in diseases such as breast cancer (Monteith et al, 2012).
Aims. To compare Ca^{2+} signaling in the inner and outer cell of CIC structures during activation.
Methods. Cells were maintained in suspension under conditions that promote the formation of CIC structures. Human breast cell lines were modified to express either the genetically-encoded calcium indicator (GECI) GCaMP or jRCaMP. The use of GECIs allowed for the observation of these CIC structures over many hours and the observation of changes in intracellular Ca^{2+} after the formation of CIC structures. Cells were stimulated using the Ca^{2+} mobilising agonist ATP and changes in intracellular Ca^{2+} were observed using confocal microscopy.
Results. A differential ATP-induced Ca^{2+} response was consistently seen between the inner and outer cell of CIC structures.
Discussion. Results from these experiments indicate that the Ca^{2+} dynamics of CIC structures are complex and differ between inner and outer cells. Whether Ca^{2+} signalling plays a role in the development and maintenance of CIC structures is now being investigated.

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Fais S & Overholtzer M (2018) Nature Rev Cancer, 18(12), 758-766.
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461 Triple negative breast cancer: Screening for the invasion amplifying cAMP-calcium feedforward loop

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Introduction. The β_2 adrenoceptor activates a cAMP-calcium (Ca^{2+}) feedforward loop in the highly metastatic triple negative breast cancer (TNBC) tumour cell line MDA-MB-231^{HM} to drive accelerated invasion (Pon et al, 2016). This loop is absent in the parental MDA-MB-231 cells and activation of the β_2 adrenoceptor does not accelerate invasion. This suggests the β_2 adrenoceptor activates unique signalling in more aggressive TNBC cells to accelerate disease.
Aims. To determine whether the β_2-adrenoceptor activates a cAMP-Ca^{2+}-invasion pathway in other TNBC tumour cells.
Methods. Formoterol was used to activate the endogenously expressed β_2-adrenoceptor in a panel of 10 TNBC cell lines. Relative mRNA expression of β-adrenoceptor subtypes was determined using qRT-PCR. Receptor signalling was measured using cAMP accumulation and Ca^{2+} mobilisation assays in the presence of a Ca^{2+} chelator (BAPTA-AM) or an adenylyl cyclase inhibitor (2',3'-dideoxyadenosine, ddA), respectively. Cellular invasion and proliferation were assessed using microscopy.
Results. There was no effect of formoterol on cAMP or Ca^{2+} in 2 TNBC cells. Activation of the β_2-adrenoceptor by the selective agonist formoterol elevated cAMP and increased intracellular Ca^{2+} in 7 out of the remaining 11 TNBC cells: HCC38 (pEC_{50} cAMP 8.58±0.44, Ca^{2+} 7.90±0.22), HCC1143 (pEC_{50} cAMP 9.88±0.33, Ca^{2+} 9.70±0.25), HCC1806 (pEC_{50} cAMP 8.88±0.48, Ca^{2+} 8.98±0.38), BT549 (pEC_{50} cAMP 9.48±0.28, Ca^{2+} 9.65±0.70), MDA-MB-468 (pEC_{50} cAMP 9.08±0.40, Ca^{2+} 9.07±0.17), HCC1395 (pEC_{50} cAMP 8.26±0.31, Ca^{2+} 7.73±0.58). BAPTA-AM and ddA inhibited cAMP and Ca^{2+} signals, respectively, suggesting that a cAMP/Ca^{2+} feedforward loop exists in these cells. Activation of the cAMP/Ca^{2+} feedforward loop correlated with accelerated invasion following β_2-adrenoceptor stimulation.
Discussion. The β_2-adrenoceptor can accelerate breast cancer progression in response to stress. The feedforward loop may provide strategies to more specifically target this GPCR in order to slow cellular invasion and metastasis.

Pon CK et al (2016) FASEB J 30:1144-1154

462 Drug repurposing screen targeting PARP in high grade serous ovarian cancer

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Introduction. Drug repurposing is a method for identifying new uses for approved drugs that are outside the scope of the original approved use. We established a drug repurposing pipeline to identify potential therapeutic strategies for chemo-resistant high grade serous ovarian cancer (HGSOC). Efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI) currently approved for the treatment of HIV infection, was predicted to have PARP-binding activity in our drug repurposing pipeline. We investigated the potential use of this drug in HGSOC cell lines.
Aims. The aims of this study were to determine the effectiveness of efavirenz using *in-vitro* models of HGSOC, and to determine whether efavirenz alters PARP expression and/or activity.
Methods. Cresset Discovery Ltd. (Cambridge, UK) used the *in-silico* ligand-based virtual screening platform *BLAZE* to identify drugs with predicted PARP-binding activity. The list of potential PARP-binding drugs was further refined by dosing and known cytotoxicity, lipophilicity, teratogenicity, and side effects (e.g.: stroke, myocardial infarction). Eight molecularly characterised HGSOC cell lines and three patient derived organoids were treated with a range of doses of efavirenz to determine cell proliferation inhibition IC_{50} values for each cell line. Response to efavirenz treatment was determined by Incucyte live-cell fluorescent assays to quantify apoptosis and cell death in real time. Western blots and PARP activity assays were performed to determine the effect of efavirenz on PARP activity and protein expression.
Results. *In-silico* BLAZE screening identified 87 drugs which were predicted to have PARP-binding activity. Further refinement led to two lead drugs being identified. Due to its pleiotropy and likely relevance to clinical dosing, efavirenz was chosen to be tested *in-vitro*. Preliminary results indicate that efavirenz treatment reduced cell confluency and induced cell death in a dose-dependent manner. Additionally, both protein expression and activity of PARP were decreased in HGSOC cell lines after efavirenz treatment.
Discussion. Based on this preliminary data, efavirenz at the doses used in this study should be further pharmacologically investigated in HGSOC.

463 Investigating the pharmacological relationship between GPR161 and spexin-1

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Introduction. GPR161 is a Family A orphan G protein-coupled receptor (GPCR) localised to a highly specialised structure of the cell, primary cilium, and involved in the negative regulation of the Sonic Hedgehog signalling pathway. Loss of GPR161 in zebrafish results in aberrant cardiac looping and in mice can lead to lumbar-sacral spina bifida, congenital cataracts and embryonic lethality. Recently, spexin-1, a 14-amino acid neuropeptide, was proposed to be a putative ligand for GPR161 (Foster et al, 2019), however, this has not yet been fully validated.
Aims. To investigate the pairing of GPR161 to the endogenous peptide, spexin-1.
Methods. RNA expression data was sourced from the Human Protein Atlas and Genotype-Tissue Expression project across 19 healthy, human tissues. Proximal interactions between GPR161 and downstream effector molecules such as G alpha (Gα) proteins and β-arrestins, and downstream extracellular signal-regulated kinases (ERK) activation were quantified using bioluminescence resonance energy transfer (BRET) assays while changes in distal second messenger levels were measured using the cyclic AMP response element (CRE) reporter gene assay, upon spexin-1 treatment. Appropriate positive controls were established for all assay systems in each individual experiment.
Results. GPR161 appears to show constitutive activity through the Gαs signalling pathway consistent with previous reports in the literature. Compared to control, no concentration-dependent change in Gα protein activation (via Gαs, Gαi1, Gαi2 or Gαi3), β-arrestin2 recruitment or ERK1/2 activation was observed for spexin-1 at GPR161. Expression of GPR161 was shown in HEK293 cells using enhanced yellow fluorescent protein (eYFP) and FLAG-tagged constructs.
Discussion. These preliminary results indicate that spexin-1 likely does not activate GPR161, despite there being some overlap in RNA expression of the two genes.

Foster SR et al (2019) Cell 179:895-908.e821

464 Assessment of Store operated calcium entry (SOCE) in replicative and accelerated models of cellular ageing

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Introduction. Calcium (Ca^{2+}) homeostasis dysregulation has been linked to many age-related diseases such as Alzheimer's, Parkinson's disease, Huntington's disease and many more. SOCE is one of the major pathways involved in the regulation of Ca^{2+} homeostasis intracellularly and it has been also linked to many age-related disorders, however the role and regulation of SOCE in cellular ageing is not clear. In this study we developed a replicative and two accelerated models of cellular senescence using hydrogen peroxide (H2O2) and Mitomycin C (MMC). We then assessed the regulation of SOCE in these models.
Aims. A. To establish an accelerated model of ageing. B. To study the regulation of SOCE in the replicative and accelerated model of ageing.
Methods. To establish an accelerated model of ageing Human derived fibroblasts (HDF) were treated with various concentrations of H202 (50 µM, 100 µM, 150 µM and 300 µM for 24 hours) and MMC (50 nM, 100 nM, 200 nM, 400 nM and 600 nM for 48 hours). Subsequently, media was replaced with normal media, and cells were further cultured for five days. Senescence was confirmed by measuring senescence associated beta-galactosidase activity using the fluorometric Spiderβ-gal assay kit and high-content microscopy. SOCE regulation in the cellular ageing models was assessed by calcium influx assays using Fluo-4 calcium indicator.
Results. There was increased activity of senescence associated beta galactosidase in the H2O2 and MMC treated HDFs when compared to control. SOCE activity was downregulated in replicative ageing, however there was no difference in the SOCE activity in the H2O2/MMC induced cellular ageing.
Discussion. We have established an accelerated model of cellular ageing using H2O2 and MMC. We also show here that SOCE is downregulated in senescent cells compared to replicating cells, however there is no change in SOCE activity in the accelerated model of ageing.

465 Screening Assays for Inhibitors against SARS-CoV-2 Spike protein and cell entry

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Introduction. The SARS-CoV-2 virus pandemic has highlighted the need for broad spectrum anti-viral therapeutics that reduce viral replication and can target current and future variants of the virus. Virus cell entry is mediated by the interaction between the spike glycoprotein and the ACE-2 cellular receptor, and virus entry can follow one of two routes (1) Fusion of the virus and cellular membranes allowing entry of virus replication machinery (Structural proteins and RNA genome) or (2) clathrin mediated endocytosis. Targeting the virus entry step with small molecule inhibitors can significantly reduce the ability of the virus to replicate and cause disease and provides an opportunity to develop therapeutics which are not hindered by virus antigenic variation or vaccine escape mutants.

In this project two assays have been developed to screen for cell entry inhibitors (A) a virus spike protein Cell fusion assay and (B) A lentiviral pseudotype assay expressing the virus spike protein. In the cell fusion assay expression of the SARS-CoV-2 envelope together with a GFP reporter gene in one cell and the expression of the ACE-2 receptor in the target cell. Co-culture and fusion of the two cell types which results in large cell syncytia and GFP expression which can be quantitated by fluorescence microscopy. In the viral pseudotype system, lentiviral particles containing the virus spike protein are produced expressing GFP or luciferase and inhibition of entry into ACE-2 target cells was assessed.

Results: Both assays were validated using known in-vitro inhibitors of viral entry including camostat mesylate, Ivermectin, proguanil hydrochloride and nitazoxanide. Screening of purified natural products highlighted inhibitory activity associated with quercetin and luteolin and inhibitory activity of medicinal plant extracts within non-cytotoxic concentrations was associated with *Pericaeta communisma*, *Dryopteridis crassirhizomatis* and *Ainsliaea acerifolia.*

Summary: A wide variety of molecules have been shown to be active in vitro against the SARS-CoV-2 spike protein and ACE-2 Receptor interaction and screening of repurposed drugs and natural products will contribute to new therapeutic candidates which may compliment currently available COVID-19 anti-virals.

466 Antiviral activity of Selective Serotonin reuptake inhibitors against enteroviruses

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Introduction. The global coronavirus pandemic has resulted in a research focus of discovery of broad-spectrum antiviral drugs. Repurposing drugs which are used for other types of treatments and have a good safety profile may provide a faster track to clinical approval. This project describes the anti-viral activity of selective serotonin reuptake inhibitors (SSRIs) which are commonly used as anti-depressants and anti-anxiety medications against the enterovirus, Human Echovirus 12. Enteroviruses are a large group of single stranded RNA viruses which include Poliovirus, Coxsackie viruses, echoviruses, and are associated with a range of illnesses including the common cold, aseptic meningitis, hand foot and mouth disease and flaccid paralysis.

Aims. To test the antiviral activity of SSRIs including Fluoxetine, Fluvoxamine, Paroxetine, Citalopram, Escitalopram and Sertraline against a model enterovirus, Echovirus 12(Travis strain).
Methods. Virus culture was performed in Vero cells and SSRIs were tested for their ability to block virus entry or to inhibit intracellular virus replication using the standard TCID50 assay together with QPCR measurement of viral RNA and immunostaining for viral double stranded RNA (dsRNA).

Results. The cytotoxicity of SSRIs was measured by the MTT cell viability assay and a concentration range of between 40uM and 5uM was used for antiviral experiments. Fluoxetine was shown to be the only SSRI with the ability to significantly reduce EV12 virus intracellular replication with an IC50 of 9.06uM, when compared to the known enterovirus protease inhibitor rupintrivir. None of the SSRIs showed inhibition at the virus entry stage in a time of entry experiment when compared to the enteroviral capsid binding inhibitor, pleconaril.

Discussion. Repurposing of currently available drugs as anti-virals may lead to the discovery of novel activity and contribute to drug design. Fluoxetine is an SSRI with good bioavailability and distribution and in this in vitro study shows potent anti-viral activity against the enterovirus, echovirus 12.

467 The search for a ligand for the pro-atherosclerotic orphan G protein-coupled receptor, GPR146

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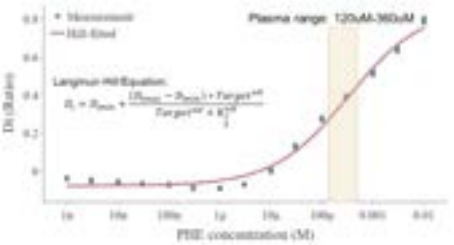

Introduction. The pro-atherosclerotic orphan G protein-coupled receptor GPR146 presents a much-needed opportunity to manage atherosclerosis in treatment-refractory sub-populations such as those with familial hypercholesterolaemia. However, the pharmacology of GPR146 remains poorly understood. It has been proposed that C-peptide, the connecting peptide of proinsulin, is the endogenous ligand for GPR146[1], although these findings have not yet been replicated. Independent studies have now demonstrated that foetal bovine serum specifically activates GPR146[1,2] and further that the active component is contained within the fraction containing species of <3 kDa in size[3].

Aims. 1) To explore the constitutive signalling profile of GPR146. 2) To validate C-peptide as a ligand for GPR146. 3) To validate the proposed activation of GPR146 by serum.

Methods. CRE luciferase, SRE luciferase, NFAT-RE luciferase, and SRF-RE luciferase reporter gene assays were used to investigate the constitutive signalling profile of GPR146 through $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$, respectively. To test the proposed ligands of GPR146, all possible canonical signalling pathways were tested using G protein reporter gene assays, NanoBiT β-arrestin recruitment assays, and YEN ERK signalling assays.

Results. No constitutive coupling to any G protein was detected. We did not find evidence that C-peptide or serum elicit specific signalling through GPR146 in any assay format investigated.

Discussion. Previous reports of C-peptide- and serum-induced activation of GPR146 were unable to be replicated. It is therefore unlikely that C-peptide is the endogenous ligand for GPR146. Given that serum is a complex biological mixture of variable composition, it is possible that the serum used in this study did not contain the active ligand. This study demonstrates the importance of independent validation of ligand receptor pairings.

[1]Yosten et al. 2013. J Endocrinol.
[2]Yu et al. 2019. Cell.
[3]Han et al. 2020. Cell Res.

468 Quantification of the inhibitory effect of neurofibromin 1 (NF1) using biosensors

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Introduction. Neurofibromin (NF1) is a crucial RasGAP for inhibiting Ras-mediated signalling. Mutations in the *NF1* gene cause an autosomal dominant disease called neurofibromatosis type 1 (NF1). The recent cryoEM structure of NF1 revealed a complicated lemniscate fold that is highly susceptible to mutation (Lupton et al. 2021). NF1 loss or dysregulation is characterised by aberrant Ras-dependent signals, such as hyperactivation of ERK, Akt and PKC and inhibition of cAMP activity. Therefore, mapping Ras-dependent signals will allow us to investigate the role of NF1 in regulating key Ras-dependent effectors and subsequent downstream signalling.

Aims. To quantify the effect of NF1 on Ras, ERK, Akt, PKC and cAMP signalling using biosensors

Methods. We developed a dual expression system for previously reported FRET-based single-fluorophore kinase biosensors. This dual biosensor expression system enables a simultaneous and multiplexed detection of NF1-regulated Ras-dependent kinase activities. FRET biosensor activity was detected using the Operetta high-content imager, BRET was detected using the PHERAstar.

Results. The dual-biosensors were transiently transfected into HEK293 cells that lack endogenous NF1 expression. NF1 co-transfection with the kinase biosensors inhibited ERK, Akt, and PKC activities, and increased the level of cAMP. Similarly, expression of NF1 inhibited both FBS- and EGF-mediated ERK activity measured using a BRET-based ERK biosensor (YEN). To directly measure the RasGAP activity of NF1, we used a HyBRET-HRas biosensor to measure H-Ras activity in basal and activated states. NF1 reduced both basal and EGF-mediated H-Ras activity.

Discussion. Overall, our preliminary findings highlight the indispensable role of active NF1 in regulating Ras-dependent signalling. Furthermore, our established screening methods using FRET or BRET have laid solid foundations for future research into the activity of NF1 mutants.

Lupton CJ (2021) Nat Struct Mol Biol 28(12): 982-988

469 Aptamer-based Phenylalanine Sensor for Cell Culture Monitoring

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Introduction. Real-time measurement of analyte concentration in cell culture medium is critical to ensure high predictability and reproducibility in drug discovery. The ability of phenylalanine (PHE) sensor has been established in vivo measurement of rats [1].
Aims. We are aiming to miniaturize and integrate this sensor in different dynamic cell culture medium supplies and compare the result between the conventional medium (CM) and the human plasma-like medium (HPLM).
Methods. The surface of the gold sensor electrode, a self-assembled monolayer (SAM) of aptamer sensors modified with a redox reporter was attached via a thiol-gold bond. The binding of PHE to the oligo probe changes the structure and dynamics of the SAM and affects the electron transfer to the gold electrode, thereby changing the observed Faradic peak current which was detected by Square Wave Voltammetry (SWV).
Results. The functional aptamer-based sensor in the experiment showed its ability to detect the concentration of phenylalanine in a molarity range between 10µM to 1mM. Especially the sensor slope in the plasma phenylalanine range (120µM - 360µM) is sufficient to identify the variation. The overall data could be fitted by Langmuir-Hill Equation with R-square greater than 0.99.
Discussion. The calibration result of aptamer-based phenylalanine sensors indicates a great potential to monitor the differences among cell culture mediums in real time. In the future, the sensor could be integrated with the pump-valve feedback control system to mimic the real cell culture environment more physiologically by precisely varying concentrations of medium nutrients.
References: [1] Idili A et al (2021b). Analytical Chemistry, 93(8), 4023–4032.

470 Investigating the atypical DRY motif of the potential lipid-lowering orphan receptor GPR146

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Introduction. GPR146 is an orphan G protein-coupled receptor (GPCR) recently linked to atherogenesis through promoting increased total serum cholesterol. It is thought act via a novel hepatic mechanism that increases very low-density lipoprotein (VLDL) secretion, and therefore may be inhibited by pharmacological agents to achieve lipid-lowering effects (Yu et al., 2019). However, the development of GPR146-targeted drugs is hampered by unknowns surrounding its function. We noted an atypical Asp-Arg-Tyr sequence in the DRY motif, which is known to regulate receptor expression and signalling. GPR146 is one of only two known receptors where arginine is substituted for a histidine, possibly presenting implications for its cellular behaviour and trafficking.
Aim. This study aims to investigate cell surface expression and constitutive signalling profiles of wild-type GPR146 and two DRY motif mutants (H3.50R, H3.50Y), where the histidine at position 3.50 is replaced either by the canonical arginine or a tyrosine residue identified in rodent orthologs.
Results. GPR146 constructs were transiently transfected into HEK293 cells and expression profile analysed by immunoblot. Whole-cell GPR146 expression did not significantly differ between DRY motif variants, and discrete receptor populations could be produced upon deglycosylation. All three variants displayed similar plasma membrane expression profiles in biotinylation pull-down studies. While previous studies have suggested coupling of GPR146 to the Gαs pathway, we report no constitutive activity of any DRY mutant through a downstream CRE-luciferase reporter assay.
Discussion. As whole-cell expression of GPR146 is equivalent across wild-type, H3.50R, and H3.50Y variants, the atypical motif is unlikely to influence receptor translation and maturation. Similarly, membrane expression is observable and broadly consistent among the constructs, suggesting that the DRY mutants examined do not influence receptor trafficking to the cell surface. However, further investigation may be warranted to confirm if this behaviour is conserved in native cell types. Finally, while GPR146 mutations did not affect constitutive Gαs GPR146 signalling, other pathways remain to be explored.

Yu, H. et al. (2019). Cell 179(6) 1276-1288.e1214.

471 The role of RunX2 in calcium-related signaling pathways in breast cancer cells

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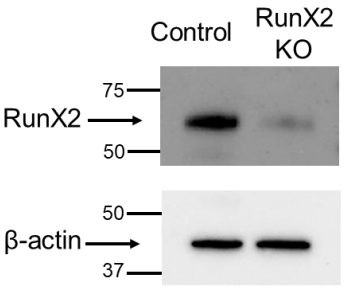
Introduction. Calcium is a second messenger regulating various cell functions such as proliferation, cell death and migration (Monteith et al, 2017). It is thus not surprising that dysregulation of calcium homeostasis is a feature of cancer (Monteith et al, 2017). A previous study has suggested a role for the TRPV1 calcium permeable channel in osteoblast formation through its regulation of RunX2 expression (He et al, 2017). In addition to its role in osteogenesis, RunX2 is also expressed in normal mammary epithelial cells, and elevated levels of RunX2 occur in human breast cancer cell lines (Lau et al, 2006). Given the interplay between calcium signalling and RunX2 during osteogenesis, studies are needed to probe the relationship between calcium signalling and RunX2 during breast cancer progression.



Aims. To generate and validate RunX2 knockdown MDA-MB-231 breast cancer cells and to identify calcium related genes regulated by RunX2 in MDA-MB-231 cells.
Methods. CRISPR/Cas9 genome editing technology was used to generate RunX2 knockdown in MDA-MB-231 cells. Genes regulated by RunX2 will be investigated by RT-qPCR and immunoblotting.
Results. RunX2 was successfully knocked down in MDA-MB-231 cells establishing a model for gene expression studies. Results suggest that RunX2 knockdown may regulate the expression of specific calcium regulators.
Discussion. RunX2 may promote the occurrence of breast cancer bone metastases (Vishal et al, 2017). Previous studies have indicated a clear interplay between calcium signalling and RunX2 in osteogenesis (He et al, 2017). Understanding the interplay between RunX2 and calcium signalling in breast cancer cell models may be the first step in the identification of potentially new molecular therapeutic targets for bone metastatic breast cancer.

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Monteith GR et al (2017) Nat Rev Cancer 17(6):367-380. Vishal M et al (2017) Int J Biol Macromol 99:608-614.

472 The influence of soluble microenvironment on tumour cell cytotoxicity of chemotherapeutics

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Introduction. There have been increasing concerns about the contrasting hypernutritional composition of conventional cell culture medium (CM) with that of the human physiological microenvironment. Melbourne medium (MM) is a plasma-like physiological medium developed by our group.
Aims. To compare the drug efficacy of breast cancer drugs in MM and CM, and to use proteomics to study protein expression differences in pathways relevant to drug mechanisms.
Methods. A systematic search for launched and discontinued breast cancer drugs was conducted using Cortellis Drug Discovery Intelligence database. Acridine orange and ethidium bromide (AO/EB) staining was used in combination with Operetta high content imaging microscope to conduct automated viable and non-viable cell numeration. The protein expression differences in MDA-MB-231 and MCF7 cells in MM vs. CM were established by global proteomics.
Results. A total of 230 discontinued drugs and 54 launched drugs were identified after the Cortellis search. The drug list was refined to 12 drugs for testing efficacy. Of the 12 drugs, paclitaxel, docetaxel and ispinesib showed differences in effectiveness in MDA-MB-231 cell line between MM and CM groups. The potency of these drugs was higher in CM compared to MM. Four out of 12 drugs also showed differences in potency in MCF-7 cell line between MM and CM groups. Global proteomics analysis indicated that 1317 and 794 proteins were differentially expressed between MM and CM in MDA-MB-231 and MCF-7 cells, respectively.
Discussion. The effectiveness of breast cancer drugs was greatly influenced by the microenvironment in which the drugs were tested. Ispinesib, a KIF11 inhibitor that failed in phase II clinical trial, showed reduced effectiveness on tumour cells in MM, a finding that if available during the preclinical evaluation may have arrested its further development.

473 Polypharmacy with high Drug Burden Index (DBI) reduces physical function with sex differences in mice.

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Introduction. Ageing, sex and medication use are associated with changes in physical function.
Aims. We investigated effects and interactions of age, sex and polypharmacy with high Drug Burden Index (DBI, total anticholinergic and sedative medication exposure) on 4 assessments of mouse physical function: automated grip strength, wire hang with 3 trials of 60s (WH60s), wire hang 1 trial of 300s (WH300s) and balance beam.
Methods. Young (2.5 months) and old (21.5 months) C57BL/6 male and female mice (n=6-8/group) were assessed for physical function at baseline, then randomised to receive either control diet or chronic high DBI polypharmacy (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin) at therapeutic doses. Following 6 weeks of treatment, mice were reassessed using the same physical function measures. Effects of polypharmacy by age and sex group and their interactions were analysed using three-way ANOVA.
Results. Pre-treatment, mice showed age differences (young performed better than old mice in all measures ($p<0.05$)) and sex differences (young males had greater grip strength than young females, old females showed greater wire hang endurance than old males (WH60s), and females traversed the balance beam faster than males ($p<0.05$)). Comparing function following high DBI polypharmacy treatment to pre-treatment, young and old males and old females showed reduced grip strength ($p<0.05$), and old females showed reduced wire hang time ($p=0.02$), while old males were not significantly affected. Overall compared to pre-treatment performance, mice treated with high DBI polypharmacy showed reduced grip strength ($p<0.0001$) and wire hang time (WH60s) ($p=0.009$). Control mice showed increased grip strength in the same 6 weeks ($p=0.0008$). In the balance beam, following polypharmacy treatment mice showed greater hindleg dragging ($p=0.0002$) indicating reduced stability with walking compared to pre-treatment, with the change most prominent in old females ($p=0.03$).
Discussion. High DBI polypharmacy treatment reduced grip strength, wire hang time and walking stability compared to pre-treatment performance. Age and sex interactions with drug effects demonstrate the importance of testing drug effects in different age and sex groups, while the full battery of behavioural tests demonstrate different sensitivity for drug/age/sex effects.

474 Hypocretin-1 receptor antagonism and inhibitory control during the go/no-go task

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Introduction. Motivation and inhibitory control are dominantly regulated by the dopaminergic (DA) and noradrenergic (NA) systems, respectively, as determined in part by use of the go/no-go task. Hypothalamic hypocretin (orexin) neurons provide afferent input to dopaminergic and the noradrenergic nuclei and hypocretin-1 receptors (HcrtR1) are implicated in reward and addiction. However, the role of the HcrtR1 in inhibitory control is not well understood
Aims. To determine the role of hypocretin-1 receptor (HcrtR1) antagonism and motivational state in inhibitory control using the go/no-go task in mice.
Methods. A HcrtR1 antagonist (BI001, 12.5 mg/kg, *per os*) or vehicle were administered 30 min before testing, once daily for 5 days, to n=23 male C57Bl/6JArc mice under high (food-restricted) and low (free-feeding) motivational states in a go/no-go task. A Latin square cross-over design was used with trained mice pseudo-randomly allocated to one of eight treatment sequences with seven-day food status transitions or three-day drug wash-out period between treatments. Linear mixed model and decision tree dendrogram were computed for behavioral data analyses.
Results. Food restriction significantly increased go accuracy, the number of non-responding presses and premature presses and decreased reaction time in the go/no-go task. Under food restriction, HcrtR1 antagonism increased no-go accuracy and decreased the number of non-responding presses. Animals were characterised into two clusters based on their performance during training with the effect of HcrtR1 antagonism more evident in the more impulsive cluster during food restriction.
Discussion. Food restriction boosted motivation of mice to achieve a better decision-making performance in the go task, while HcrtR1 antagonism increased go and no-go performance, decreased impulsivity and increased inhibitory control in impulsive mice only.

475 Desensitising Properties of Gain-of-function *GABRB3* variants influence the severity of DEE

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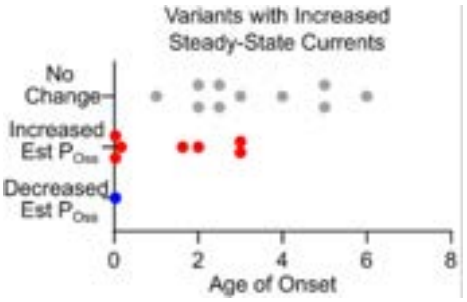
Developmental and epileptic encephalopathies (DEE) are characterised by seizures beginning in childhood. Many are associated with genetic variants in the *GABRB3* gene, that encodes for the β3 subunit of $GABA_A$ receptors ($GABA_AR$). These variants can alter GABA sensitivity resulting in either a gain (GoF) or loss of receptor function (LoF). A recent genotype/phenotype correlation study of DEE-causing *GABRB3* variants determined that patients with GoF variants have a distinct and more severe clinical phenotype than those with LoF variants (1). It is unclear whether receptor desensitisation contributes to the severity of the patient phenotype.
Aim: To determine how $GABA_AR$ desensitisation influences the severity of patient phenotype.
Method: 20 GoF and 5 LoF variants were expressed in *Xenopus oocytes* as singly mutated concatenated receptors. Current decay rates, steady-state currents (Est. Po(ss, max)) and maximum open probability (Est. Pomax) were measured using two-voltage clamp electrophysiology. Data were compared with non-parametric ANOVA and Dunn's posthoc test. Comparisons were restricted to data from variants measured on a single day.
Result: GoF variants that increased the current decay typically had an older onset (age of onset > 4 months), meaning receptors were more desensitised than wild-type (WT). However, the most severely affected patients (age < 2 months) with GoF variants had, in general, a significant increase in Est. Po(ss,max) compared to WT, meaning receptors were less desensitised. Whilst GoF variants did not alter the Est. Pomax, all 5 LoF variants had a significantly lower Est. Pomax ($p < 0.05$).
Conclusion: Variants that resulted in receptors with reduced desensitisation and increased GABA sensitivity are likely to exacerbate the severity of patient phenotype.



1. Absalom NL et al Nat Commun. 2022;13(1):1822.

476 Investigating an orexin receptor 2 structure-function relationship with C-terminus mutants.

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Introduction. While the function of the orexin system is established as mediating arousal and appetite, the divergence of the molecular function between the two orexin G protein coupled receptors (GPCRs); orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R), is to be delineated. Serine/threonine phosphorylation sites along the OX_2R C-terminus are known to be crucial for recruiting β-arrestin following activation of the receptor (Jaeger et al, 2014). This project employed mutant OX_2Rs with alanine-substituted phosphorylation sites along the C-terminus to further investigate the function of these sites.
Aims. To determine if phosphorylation sites designated 399, 406 and 427 along the OX_2R C-terminus may inform a pharmacological structure-function relationship characteristic of OX_2R.
Methods. Bioluminescence resonance energy transfer (BRET) assays which assessed the activation of $G\alpha_i$, $G\alpha_q$ and $G\alpha_s$, and the recruitment of β-arrestin2, were conducted with five OX_2R constructs; Wild-type, Δ406, Δ399-427, Δ406-427 and Δ399-406-427, in response to stimulation by orexin A (OxA) and orexin B (OxB).
Results. Reduction in β-arrestin2 recruitment to the cell membrane was the most significant effect of C-terminus phosphorylation site alanine substitution. OxA and OxB concentration-response assays for $G\alpha_q$ activation reveals leftward potency shifts in the alanine-substituted OX_2R constructs. Some alanine-substituted constructs appear to have altered $G\alpha_i$ and $G\alpha_s$ activation compared to wild-type, however, mostly failed to reach statistical significance.
Discussion. A molecular structure-function relationship that would serve to delineate the functions of the two orexin receptors is unlikely to be explained solely by C-terminus phosphorylation sites. Agreeing with the outcomes of a previous study which looked at these alanine-substituted constructs, the complex tertiary structures formed by the intracellular domains are more likely to influence the molecular pharmacology of OX_2R (Jaeger et al, 2014).

Jaeger WC et al (2014) Br J Pharmacol 171:364-374

477 Role of endocannabinoids in fear learning in a murine neuropathic pain model.

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Introduction. Chronic neuropathic pain is a highly prevalent and disabling condition characterised not only by sensory abnormalities, but also major psychological comorbidities. Currently, psychological interventions that manage neuropathic pain, are limited by their accessibility and efficacy. There is evidence that the endogenous cannabinoid (endocannabinoid) system has a crucial role in fear learning. Thus, drugs that target the endocannabinoid degradation enzymes, fatty acid amino hydrolase (FAAH) and monoacylglycerol lipase (MAGL) are thought to be promising therapeutic targets. However, the effect of these endocannabinoid drugs on fear related neuropathic pain maladaptations are unknown.
Aims. To examine the effect of nerve-injury induced neuropathic pain on fear learning and extinction, and whether this is altered by endocannabinoid modulatory drugs.
Methods. Adult male C57BL/6 mice underwent either no surgery (naïve), control sham surgery (shams), or sciatic nerve chronic constriction injury surgery (CCI) (*n* = 8/group). Experiments were performed 14 days after surgery. Fear learning and extinction were assessed via standard Pavlovian conditioning, with a footshock (0.9 mA, 1s duration) paired with an audio tone (5 kHz, 30s duration) on day 1, and the audio tone only on days 2-4. Fear behaviour was quantified as the percentage time spent freezing. Some animals received a subcutaneous injection of either vehicle, or the endocannabinoid degradation enzyme inhibitor JZL195 (3mg/kg) on days 2-3.
Results. Freezing on days 2-3 was not significantly different between naïve, sham and CCI animals. Freezing on day 4 was more in CCI, compared to naïve and sham animals. JZL195 abolished the difference in day 4 freezing between naïve, sham and CCI animals.
Discussion. These results indicate that fear extinction, but not fear learning is disrupted in the nerve-injury model of neuropathic pain. Blockade of endocannabinoid degradation appears to reduce the disruption of fear extinction in the neuropathic pain state. Thus, endocannabinoid modulatory drugs could be used to help with neuropathic pain psychological interventions.

479 Exploring cardiac GPCRs for effective fibrosis treatment

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Introduction. Pathological fibrosis in the myocardium causes cardiac remodelling leading to heart failure and serves as a converging point for almost all heart diseases. There remains unmet therapeutic need to treat cardiac fibrosis in order to reduce heart failure progression. The second messenger cAMP, commonly modulated downstream of G protein-coupled receptor (GPCR) activation, has been shown to inhibit cardiac fibrosis may be harnessed as a good therapeutic target in heart failure.
Aim. This study was conducted to evaluate GPCR modulation of cAMP signaling in adult human ventricular cardiac fibroblasts (NHCF-V) in the presence or absence of the pro-fibrotic mediator, transforming growth factor beta 1 (TGF-β1).
Methods. This study evaluated 11 candidate GPCRs, selected based on gene expression in both human cardiac fibroblasts (The Human Protein Atlas) and human cardiac organoid. GPCR-mediated cAMP accumulation in NHCF-V was evaluated with LANCE cAMP assay. NHCF-Vs were treated with 1 µM forskolin and then exposed to agonists of selected GPCRs for 30 min in the absence or presence of 48 hr TGF-β1 (10 ng/mL) pre-treatment. Nonlinear regression in GraphPad Prism 9.3.1 was used to quantify the potency (pEC50) of the agonists. Data are presented as mean ± SEM.
Results. Increased cAMP accumulation was observed in NHCF-V upon stimulation with 5'-N-ethylcarboxamidoadenosine (pEC50 = 7.2 ± 0.6; + TGFβ-1 pEC50 = 6.9 ± 0.4), isoprenaline (pEC50 = 8.1 ± 0.8; + TGFβ-1 pEC50 = 8.3 ± 1.0), beta calcitonin gene-related peptide (pEC50: not determined) and histamine (pEC50: not determined), effects predicted to be mediated by the A_{2B} receptor, β_2-adrenoceptor, calcitonin receptor-like receptor and H_2 receptor, respectively. A reduction in cAMP accumulation was observed upon stimulation with ozanimod (pEC50: not determined) and spexin-1 (pEC50 = 9.1 ± 0.3; + TGFβ-1 pEC50 = 9.4 ± 1.1), effects predicted to be mediated by $S1P_1$ receptor and G protein-coupled receptor 161, respectively.
Discussion. These findings have quantified GPCR-mediated modulation of cAMP accumulation, a pathway pertinent to fibrosis, in human cardiac fibroblast in the absence and presence of the profibrotic cytokine TGF-β1.

480 Intermittent Fasting Protects Against Chronic Cerebral Hypoperfusion-Induced Cerebrovascular Transcriptomic Changes.

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Introduction. Chronic cerebral hypoperfusion (CCH) is a key mechanism that has been shown to contribute to the pathogenesis of vascular cognitive impairment and dementia. The heterogeneous effects of CCH means that single pharmacological agents are unlikely to be effective. Intermittent fasting (IF) modulates numerous signalling pathways and has been shown to be neuroprotective across a range of disease conditions including stroke, but its effects against CCH-induced pathologies remain to be elucidated.
Aims. To determine the effect of IF on CCH-induced cerebrovascular damage.
Methods. Male C57BL/6 mice were subjected to either ad libitum feeding (AL) or IF (16 hours of fasting per day) for 4 months. In both groups, CCH was experimentally induced by the bilateral common carotid artery stenosis (BCAS) method. Sham operated groups were used as controls. We assessed physiological parameters and transcriptomic changes in cerebral arteries 1, 14, 21 and 30 days after BCAS surgery.
Results. IF mice had significantly lower body weight and plasma glucose whereas plasma ketones were elevated compared with AL mice ($P<0.05$). We isolated cerebrovascular RNA for whole genome sequencing. In AL mice, genes related to angiogenesis and inflammation were differentially expressed 1 day after BCAS. The profile of the transcriptome changed over the 30 days with differential expression of genes related to cellular stress response (14 days after BCAS), histone modification, regulation of cytokines and inflammation (21 days after BCAS) and vascular structure and inflammation (30 days after BCAS). In IF mice, transcriptomic changes induced by BCAS were altered compared with AL mice. For example, we observed fewer differentially expressed genes relating to cellular stress, inflammation and histone modification.
Discussion. Our analysis of the cerebrovascular changes induced by IF and BCAS reveals novel molecular profile that may lead to the identification of key pathways that protect the vasculature during CCH.

481 Use of oral effective dose 50 (ED50) to optimise antihypertensive drug dose

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Introduction. Drug efficacy plateaus with increased dose, whilst the incidence of multiple adverse events (AEs), which with antihypertensives includes cerebral, myocardial and renal ischaemia, continues to increase. Higher risk patients may merit higher doses but in the largest randomised clinical trials (RCTs) no combination antihypertensive regimen has lowered systolic blood pressure (SBP) long term by more than 20 mm Hg[1]. The largest reductions in cardiovascular and total mortality (which usefully summate efficacy and safety) have been observed with combinations of diuretics, beta-blockers and vasodilators, and with SBP reductions of less than 10 mm Hg.
Aims. To define optimal dose ranges for antihypertensive drugs, based around effective dose 50 (E50), the mean population oral dose that causes a reduction in SBP of 50% of the maximum possible effect (Emax).
Methods. Estimates of near maximum SBP lowering (which approximate Emax) with different antihypertensive drug classes were established based on published findings from Cochrane systematic reviews of the relevant RCTs.
Results. Emax and (ED50), mm Hg, for thiazide diuretics appear to be 12 (6), beta-blockers 10 (5), ACE-inhibitors 10 (5), angiotensin receptor blockers 12 (6) and calcium channel blockers 14 (7). BP reductions begin to plateau below ED50.
Discussion. AEs, some lethal, increase with dose[2] and may explain the reduced mortality benefit with higher doses. Antihypertensive drugs when used in combination are effective (and safer) at doses below ED50.

1 Dahlof B (1991) Lancet 338: 1281-5
2 Dimmitt S (2019) Brit J Clin Pharm 85: 2218-27

482 Unravelling the intracellular signalling pathways in CGRP-induced vasorelaxation in rat coronary arteries

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Introduction. Calcitonin gene-related peptide (CGRP) may play an important role in modulating coronary microvasculature resistance and is known to be cardioprotective. However, despite its clinical importance, the precise signalling pathways underlying vasodilation are yet to be elucidated in these vessels.
Aims. To establish the role of endothelial cells (ECs) and smooth muscle cells (SMCs) and the signalling pathways activated during CGRP-induced vasorelaxation in the coronary microvasculature.
Methods. Coronary septal arteries were isolated from male Wistar rats and mounted in the wire or pressure myograph at 37°C and left to develop myogenic tone. ECs were removed with a hair and confirmed by <10% relaxation to 1 µmol/L ACh. Arteries were pre-incubated with inhibitors for 15 minutes prior to CGRP application. For EC Ca^{2+} imaging, ECs were selectively loaded with Oregon Green BAPTA and Ca^{2+} activity was recorded using a linescan confocal microscope. EC Ca^{2+} activity was analysed using Imaris. Data were analysed using either parametric two-way ANOVA with Sidak's multiple comparisons or paired t-test, or non-parametrically using Mann-Whitney with multiple comparisons followed with Bonferroni-Dunn correction. $p < 0.05$ was considered statistically significant.
Results. CGRP (1 pmol/L–10 nmol/L) caused a concentration-dependent relaxation (EC_{50} 50 pmol/L, n=6) that was right-shifted by the nitric oxide synthase inhibitor, L-NAME (100 µmol/L, EC_{50} 1.4 nmol/L, n=6), and removal of the endothelium (EC_{50} 1.6 nmol/L, n=14). The Gβγ-subunit inhibitor, gallein (100 µmol/L), also significantly attenuated vasorelaxation to CGRP (EC_{50} 50 nmol/L, n=5). No global increase in EC intracellular Ca^{2+} was observed (F/F0 = 0.98±0.05, n=5) following CGRP application in ECs loaded with Ca^{2+} indicator, but it was observed in response to ACh (F/F0 = 1.28±0.02, n=5).
Discussion. This study indicates that the most potent CGRP-induced vasorelaxation in rat septal arteries is EC-dependent. EC dysfunction in the coronary microvasculature underlies several pathological conditions and results in the reduced bioavailability of nitric oxide; therefore, this could attenuate the vasodilator action of CGRP on coronary vessels in these patients. Interestingly, it appears that CGRP-induced nitric oxide release relies on a Ca^{2+}-independent pathway. Furthermore, these data suggest that both the EC-dependent and -independent components to CGRP vasorelaxation occur via a Gβγ-mediated signaling pathway in rat coronary arteries.

483 Australian patients' perspectives on oral anticoagulants initiation in atrial fibrillation: A qualitative descriptive study

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Introduction. Patient refusal has been reported to contribute to oral anticoagulant (OAC) non-prescription in patients with atrial fibrillation (AF).
Aims. This study aimed to explore barriers and facilitators of patient acceptance of OAC initiation from the perspectives of patients with AF attending Australian general practices.
Methods. A qualitative descriptive study was conducted from March to July 2022. The interview guide and data analysis were informed by the Theoretical Domains Framework (TDF) and the Capability, Opportunity, Motivation-Behaviour (COM-B) model.

ID	State	Sex	Age	CHA2DS2-VASc score
P1	WA	M	81	4
P2	NSW	M	78	4
P3	WA	M	74	5
P4	WA	M	99	3
P5	WA	F	72	3
P6	NSW	M	77	4
P7	WA	M	81	3
P8	WA	F	79	3
P9	NSW	F	74	3
P10	NSW	F	85	4

Results. Ten patients participated in a semi-structured interview (60% male, median age = 78.5 years). All three components of the COM-B model and 10 of the 14 domains of TDF were identified to influence patient acceptance of OAC initiation. The passive role of patients in decision-making was identified as a facilitator. The majority of patients reported that they do as advised by their doctors, and hence, accept OAC initiation. Other prominent facilitators included alignment of recommendation with patients' overall health goals including prevention of stroke and associated disabilities, adequate explanations from doctors, and clear understanding of the pros and cons of taking OACs. Reportedly inadequate explanation from doctors and the inconvenience associated with taking warfarin were identified as potential barriers.
Discussion. While patients with AF usually play passive roles in thromboprophylaxis decision-making, alignment of recommendation with patients' overall health goals, adequate explanations from doctors, and a clear understanding of the pros and cons of taking OACs affect their decision to commence OACs that are recommended by their doctors.

484 Effects of acute and chronic trimethylamine-N-oxide and simulated diabetes in cardiac myoblasts

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Introduction: Trimethylamine-N-oxide is a gut metabolite receiving considerable attention for its proposed involvement in cardiovascular disease (CVD). However, whether elevated TMAO reported in cardio-metabolic disorders reflects causal involvement, or a secondary or biomarker role in disease, remains to be elucidated. The cardiovascular, metabolic and energetic effects of TMAO warrant study in models of disease.
Aims: To characterise the effects of TMAO on cell viability and death, mitochondrial respiration, and gene expression in cardiomyoblasts under control conditions and in simulated diabetes mellitus (SDM).
Methods: H9c2 cardiomyblasts were maintained under control (5 mM glucose, 0 mM palmitate, 0 mM insulin) or SDM conditions (25 mM glucose, 100 µM palmitate, 100 nM insulin) and incubated with 10 or 100 µM TMAO for 1 or 72 hrs (acute and chronic exposures). Cells were also grown for 1, 5, and 10 hrs with and without similar TMAO concentrations under control conditions. Cell viability and death were assessed via an MTT assay and extracellular protein (LDH) release, with mitochondrial respiration detailed using an Oroboros Oxygraph-2k system. Changes in myoblast gene expression were also assessed at the 1 and 72 hr time points via quantitative real-time PCR.
Results: Simulated diabetes alone worsened cell viability and death, and significantly reduced mitochondrial respiration. In contrast, acute exposure to 10-100 µM TMAO did not influence viability, death or respiration (in healthy or SDM cells). However, mitochondrial respiration in both healthy and SDM cells was significantly repressed by more prolonged incubation with 100 µM TMAO. Expression of genes related to mitochondrial fission processes were reduced with SDM but unaltered by TMAO.
Discussion: *In vitro* studies indicate that while diabetic conditions detrimentally impacts cardiomyoblast viability and death, TMAO fails to influence these measures (in healthy or SDM cells). However, mitochondrial respiration is reduced during prolonged exposure to TMAO, an effect exaggerated by SDM. These findings provide some support for a potentially causal role for TMAO in cardio-metabolic disease, though further investigation is needed to further test such effects and unmask underlying molecular mechanisms.

485 Influence of natriuretic peptides on vasculature– Is cell culture a valid approach for drug testing?

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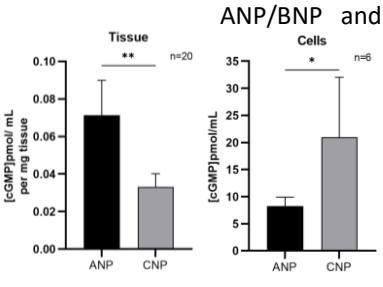
Introduction. Natriuretic peptides (NPs: ANP, BNP and CNP) recently gained attention as promising targets for the therapy of cardiovascular disease. NPs induce vasodilation of arterial vessels by binding specific receptors which in turn release the smooth muscle cell (SMC) relaxing molecule cGMP.
Aim is to evaluate whether primary aortic SMCs sufficiently reflect the functionality and expressional landscape of the NP receptors in the intact blood vessel for use in testing future therapeutics.
Methods. SMC layer (tunica media) of rat aortae were prepared and halved longitudinally. One half was left intact for experiments whereas the other half was used to isolate SMCs for culture via enzymatic digest. NP receptors expression in corresponding tissue and cells was determined via RT-qPCR. Activity of ANP/BNP and CNP receptors was revealed by ELISA measurement of the cGMP release after NP treatment for 30 min (figure: Pairwise comparison evaluated by t-test. Data shown +SD. Significance level indicated as * $p<0.05$, ** $p<0.01$).
Results. Within the intact tissue the ANP/BNP receptor was found to be expressed higher than the CNP receptor, whereas in cultured SMCs it was vice versa. Regarding the NP/cGMP system, the NP receptors where not the only key players with an altered expression pattern in cell culture. In addition, the switch in ANP/BNP and CNP receptor expression was confirmed functionally by ELISA. Here the ANP treatment of intact tissue resulted in a higher cGMP release than CNP (~ 50%), whereas in the primary SMCs (passage 2) treated with CNP the cGMP level was higher (~60%) (figure).



Discussion. Our data shows that isolation and culturing affect the expression of NP receptors and cGMP related genes in primary vascular SMCs. Hence, *in toto* approaches might reflect NP related signalling more realistically. A reason could be a general expression switch of all SMCs when cultured. The question remains whether such a drastic change in cultured cells is a special feature of the NP/cGMP signalling pathway?

486 Sex differences in risk of diabetes-associated cardiovascular events with SGLT2i versus GLP-1Ras

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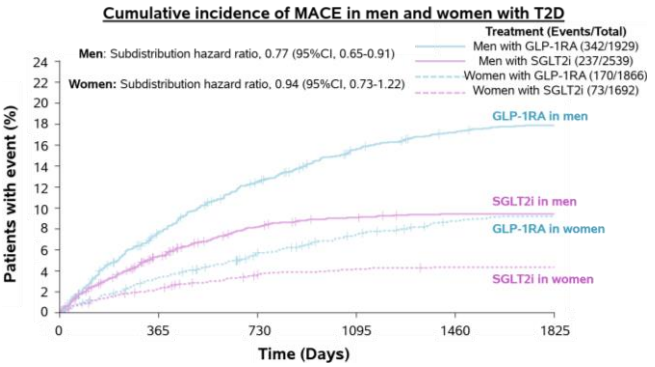
Introduction. Despite known sex differences in diabetes-induced cardiovascular disease, pharmacological treatment recommendations are independent of sex. Sodium glucose co-transporter-2 inhibitors (SGLT2i) and glucagon-like peptide 1 receptor agonists (GLP-1RAs) reduce major adverse cardiovascular events (MACE) in people with type 2 diabetes (T2D), however their potential sex-specific effects remain unknown.

Aims. The objective was to test the hypothesis that sex-specific differences in patient MACE outcomes are evident in SGLT2i vs. GLP-1RA-prescribed T2D Australians.



Methods. This population-based cohort study included men and women with T2D (≥30 years), discharged from a Victorian hospital between 2013 and 2017, and dispensed an SGLT2i or GLP-1 RA within 60 days of discharge (n=8026, 44.3% women). Using Cox proportional hazards regression with Fine and Gray competing risks, subdistribution hazard ratios (sHR) with 95% confidence intervals (CI) were estimated for MACE, its individual components, and mortality in a follow-up to mid-2018. Analyses were conducted for all men and women, further stratified by age and baseline heart failure (HF) status.

Results. In a median follow-up time of 756 days, SGLT2i (n=4231) vs. GLP-1RAs (n=3795), reduced the rate of MACE in men (sHR 0·75; 95%CI 0·64-0·89), but not women (figure). SGLT2i reduced MACE rates in men (sHR 0·64; 95%CI 0·47-0·87) and women (sHR 0·48; 95%CI 0·29-0·81) ≥65 years, and in men with baseline HF (sHR 0·45; 95%CI 0·28-0·73).

Discussion. SGLT2i, relative to GLP-1RAs, demonstrate favourable effects for the reduction of MACE among Australian men, including older men, and men with baseline HF. Analogous benefit in women is only evident in older females.

487 Antiarrhythmic effects of phenytoin, a novel heart failure diastolic ryanodine channel inhibitor, in human failing ventricle.

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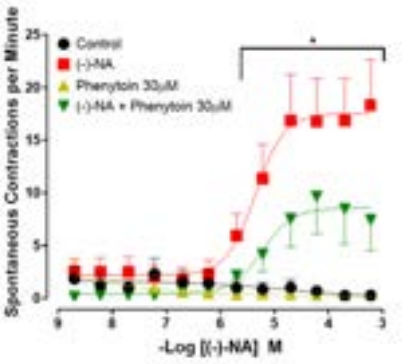
Introduction. Phenytoin, a hydantoin derivative, was recently discovered to inhibit diastolic Ca^{2+} leak from cardiac ryanodine receptor (RyR2) channels of human failing hearts. Phenytoin functions without adversely inhibiting Ca^{2+} release during systole or impacting normal Ca^{2+} RyR2 mediated release from healthy hearts (Ashna et al., 2020).

Aims. To determine the effectiveness of phenytoin to prevent arrhythmic contractions 1. in a human model of ventricular arrhythmia, 2. in hearts from β- and non β-blocked heart transplant patients.

Methods. 15 explanted ventricles with advanced heart failure were used to determine antiarrhythmic properties of phenytoin.



Results. In human failing ventricular trabeculae, 30 μM phenytoin, reduced spontaneous beats stimulated by (-)-noradrenaline (NA). Subgroup analysis revealed phenytoin did not affect spontaneous contractions stimulated by NA in patients administered β-blockers (101/10), however reduced spontaneous contractions in patients without chronic β-blocker administration (49/5). N values displayed as (trabeculae/hearts).

Discussion. We conclude that phenytoin, with known RyR2 stabilising capabilities, significantly reduces spontaneous beats in ventricular trabeculae from human failing hearts. We hypothesise phenytoin's mechanism is likely dependent on modifications of the RyR2 in heart failure, which may be reversed by chronic β-blocker administration. Further studies are required to prove mechanism of action.

Ashna A et al 2020 Mol Pharmacol, 97(4), 250

488 Gene expression analyses of Tas1R taste receptors in cardiometabolic disease

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Introduction. The sweet taste receptor (STR) is a family C G protein-coupled receptor responsible for cellular responses to sweet stimuli. The functional heterodimer, consisting of the TAS1R2 and TAS1R3 subunits, localises with the G protein α-gustducin in taste-sensory epithelium. It is commonly reported that these elements of the STR signaling system are present in cardiometabolic tissues, suggesting a role in nutrient sensing and metabolic regulation.
Aims. To use publicly available datasets to compare STR mRNA expression in human pancreas, intestine and adipose tissue, and to determine whether this expression is likely physiologically significant. Next, to identify diseases associated with the STR genes and examine changes in expression in diseased models. Finally, to examine co-expression of taste receptor genes and signaling partners in these same extraoral tissues.
Methods. Human gene expression data was mined from bulk tissue and single cell RNA-sequencing studies. Sequencing counts were extracted as transcripts per million RNA reads (TPM) for TAS1R2, TAS1R3, GNAT3 and control genes including: (1) marker genes ubiquitously expressed in the specific tissues tested, (2) other GPCRs with known physiological roles in the specific tissues tested, (3) other GPCRs with known expression outside the tissues of interest. Expression values of the STR were then compared to studies modelling diabetic tissue expression and rodent tissue expression to account for changes that may be observed in these conditions. An aggregate co-expression network from multiple tissues was then used to assess local and global gene set connectivity between taste-related genes and other functional GPCRs in the tissues of interest.
Results. Gene expression of STR signalling elements in human cardiometabolic tissue rarely exceeded 0.5 TPM, which was negligible when compared to physiologically active genes. This same result was observed in diseased tissues. In rodents, expression of the STR was marginally higher. Taste genes did not show significant co-expression in the extraoral tissues.
Discussion. We found scant evidence for physiologically-relevant STR gene expression levels in human cardiometabolic tissue, suggesting that the STR is unlikely to be a promising target for the treatment of cardiovascular and metabolic diseases.

489 Metabolic profiling of mice with deletion of orphan GPCR, GPR37L1

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Introduction. The physiological role for GPR37L1, an orphan G protein-coupled receptor, is not well understood. GPR37L1 has a known role in cardiovascular homeostasis (Coleman et al, 2018; Mouat et al, 2021), and pilot data from our laboratory indicated that *Gpr37l1* knockout mice had elevated body weight between 6-12 months of age.
Aims. This study aimed to thoroughly characterise the metabolic phenotype of mice with genetic deletion of *Gpr37l1* to determine how this receptor may be affecting whole body energy homeostasis.
Methods. Male *Gpr37l1*$^{-/-}$ mice and wildtype littermate controls (C57BL/6J) were subjected to either 12 weeks of high fat diet (HFD) or standard chow (9-21 weeks of age), or 1 year of chow diet (9-52 weeks of age), with body composition quantified by EchoMRI, glucose handling by glucose and insulin tolerance tests, and energy expenditure by indirect calorimetry. Results analysed by two-way ANOVA or ANCOVA, with Holm-Sidak post-hoc test.
Results. HFD robustly induced obesity and impaired glucose tolerance in wildtype and *Gpr37l1*$^{-/-}$ mice to a similar degree. Both HFD- and chow-fed *Gpr37l1*$^{-/-}$ mice had an elevated respiratory exchange ratio during daylight hours (7am-7pm; genotype effect P=0.036), indicating an energy production bias towards glycolytic metabolism. Further, there was a trend towards elevated overall energy expenditure by *Gpr37l1*$^{-/-}$ mice during the night period (genotype effect P=0.057). This is consistent with the aged cohort; lower fat mass was observed in *Gpr37l1*$^{-/-}$ mice at 52 weeks of age when compared to wildtype (genotype effect P=0.031), which occurred despite lower ambulatory activity in *Gpr37l1*$^{-/-}$ subjects (genotype effect P=0.017).
Discussion. Minor changes in energy metabolism seen in young *Gpr37l1*$^{-/-}$ mice are likely to contribute to the reduced accumulation of fat mass seen in the aged cohort. This study provides evidence for a modest role of GPR37L1 in whole body energy homeostasis.

Coleman JLJ et al (2018) Biol Sex Differ 9: 14
Mouat MA et al (2021) Front Pharmacol 11: 2384-2404

490 High-definition flow cytometry of the mouse kidney for single cell studies.

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Introduction. Chronic kidney disease (CKD) is on the rise throughout the world and its pathophysiology is complex likely, a reflection of the broad cellular heterogeneity of the kidney. However, the precise renal cellular changes during CKD are not well-understood. While recent advances in single-cell technologies such as flow cytometry and single-cell RNA sequencing (scRNAseq) have provided opportunities to study the renal cellulome, there is disparity in the field from variations in single-cell preparation protocols.
Aims. To identify differential kidney cellular heterogeneity in three widely used protocols for single-cell preparation.
Methods. Single-cell suspensions were prepared from male and female C57BL/6 kidneys using the following mechanical and enzymatic dissociation protocols: (1) a highly published multi-tissue digestion kit from *Miltenyi (*Park *et al*., 2018); (2) a scRNA-seq protocol (*Pinto*)(McLellan *et al*., 2020); and (3) the currently optimized protocol from the *CCBDR* laboratory (Krishnan *et al*., 2019). Following dissociation, cell suspensions were stained with antibody panels to identify major known kidney cell types: leukocytes (myeloid-derived and lymphocyte subtypes), smooth muscle cells, endothelial cells, pericytes, podocytes, fibroblasts, epithelial cells, and epithelial subtypes of the nephron.
Results. High-dimensional flow cytometry revealed that all three protocols produced a similar proportion of viable cells. Interestingly, the *Miltenyi* protocol yielded significantly less leukocytes compared to the other two techniques. The *Pinto* and *CCBDR* protocols produced similar yields for most cell types except, the *Pinto* method significantly enriched endothelial and myeloid-derived cells whereas, the *CCBDR* protocol enriched epithelial cells.
Discussion. Future single-cell studies that aim to enrich for specific kidney cell types, may benefit from this comparative analysis

491 Impairment of prostacyclin signalling: impact of ageing and coronary spasm

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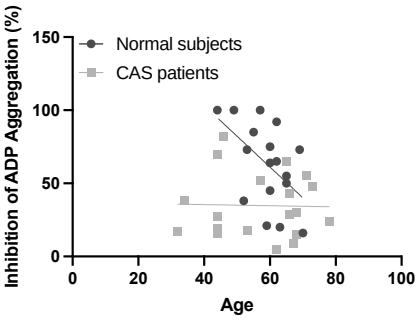

Introduction. Cardiovascular ageing is associated with progressive attenuation of vasodilator and anti-aggregatory responses to nitric oxide (NO), and there is increasing evidence that this "NO resistance" is a marker of increased risk of cardiovascular morbidity and mortality. We have also shown that NO resistance may appear in platelets at younger ages in patients with ischaemic heart disease and heart failure, but especially in patients with coronary artery spasm (CAS). On the other hand, less is known regarding changes in signalling with other anti-aggregatory autacoids such as prostacyclin (PGI_2) in either normal ageing or CAS. We therefore examined changes in anti-aggregatory responses to the NO donor sodium nitroprusside (NO) and to the PGI_2 analogue Iloprost (IP) with normal ageing and in association with the chronic phase of CAS.
Methods. Platelet aggregation was induced in whole blood with ADP and anti-aggregatory responses to SNP and IP determined.
Results. Normal ageing (17) was associated with substantial attenuation of anti-aggregatory responses to SNP and IP ($p<0.001$ for both). CAS (n=21) was associated with markedly impaired responses to both SNP and IP ($p<0.01$ for both), but with a minimal rate of age-related decline relative to that seen with normal ageing (ANCOVA: $p=0.02$ for both). With normal ageing, the ratio of IP:SNP responses tended to increase.
Conclusions. Normal ageing is associated with progressive and substantial declines in platelet responsiveness to the anti-aggregatory effects of both NO and PGI_2, and it is possible that with advanced age, PGI_2 represents the prominent homeostatic anti-aggregatory autacoid. On the other hand, the current results demonstrate that CAS should be regarded as a condition whereby both the NO and PGI_2-initiated signalling pathways are severely attenuated, even with early age of onset.

492 Challenging the inhibitory/excitatory paradigm: Increased GABAergic inhibition can lead to epilepsy

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Introduction. Patients with developmental and epileptic encephalopathies (DEE) can present with variants in genes that code for $GABA_A$ receptors. Historically, these variants are presumed to cause loss-of-function receptors leading to reduced neuronal GABAergic activity. Yet, patients with $GABA_A$ receptor variants have diverse clinical phenotypes and many are refractory to treatment despite the availability of drugs that enhance GABAergic activity. Aims. To evaluate the function of *GABRB3* or *GABRD* missense variants identified from patients with DEE. Methods. Two-electrode voltage clamp methods were used to assess the function of 44 pathogenic *GABRB3* and 6 pathogenic *GABRD* missense variants. Phenotype/genotype analysis was used to determine patient phenotype correlations. Results. We show that variants segregate into gain-of-function and loss-of-function groups. Respective patients display distinct clinical phenotypes. The *GABRB3* gain-of-function cohort (n = 27 patients) presented with a younger age of seizure onset, higher risk of severe intellectual disability, focal seizures at onset, hypotonia, and lower likelihood of seizure freedom in response to treatment. Febrile seizures at onset are exclusive to the loss-of-function cohort (n = 47 patients) (1). Of the *GABRD* cohort, 5 patients displayed a gain-of-function variant and these patients suffered from generalized epilepsy and various degrees of learning difficulties or intellectual disability, whereas the one patient with a loss-of-function variant had autism spectrum disorder, normal cognition and no seizure history (2). Discussion. Overall, patients with *GABRB3* and *GABAD* variants that increase GABAergic activity have more severe developmental and epileptic encephalopathies. This paradoxical finding challenges our current understanding of the GABAergic system in epilepsy and how patients should be treated.

1. Absalom et al 2022, Nat Commun, 13(1):1822
2. Ahring et al 2022, Brain, 145(4):1299-1309

493 Pirfenidone is more effective than glucocorticosteroids in limiting influenza-A-viral infection and inflammation.

Julia G Chitty[1], Bruce Montgomery[2], Jack A Elias[3], Philip Bardin[4,5], Jane E Bourke[1], Belinda J Thomas[5]. [1]Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, [2]Avalyn Pharma, SEA, WA, USA, [3]Division of Biology and Medicine, Brown University, RI, USA, [4]Monash Lung and Sleep, Monash Medical Centre, Clayton, VIC, and [5]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC.

Introduction & Aims. Patients with respiratory diseases are more susceptible to viral infection, often developing more severe and prolonged symptoms which often lead to deleterious effects such as lung function decline and irreversible disease progression. Emerging evidence suggests elevated transforming growth factor-beta (TGFβ), seen in patients with respiratory diseases, likely plays a crucial immunosuppressive role to enhance viral infection. While glucocorticosteroids (GCS) effectively reduce inflammation, substantial evidence demonstrates their immunosuppressive effects. Pirfenidone (PFD) is an anti-fibrotic small molecule used in patients with pulmonary fibrosis to slow disease progression. Our recent studies have shown oral administration of PFD can reduce TGFβ-enhanced viral infection in a mouse model. However, the use of oral PFD comes with unpleasant side effects, therefore the aim of this study was to determine if inhaled PFD could also reduce inflammation and disease severity and compare its effectiveness to that of GCS.
Methods. Transgenic C57Bl/6 mice with inducible lung-specific over-expression of TGFβ were treated intranasally with vehicle (control), PFD (13.3 mg/kg) or GCS (1 mg/kg) daily, starting 2 days prior to infection with IAV (102 PFU, HKx31). Mice were culled at day 3 post infection, and lung tissue and bronchoalveolar lavage fluid (BALF) were collected for assessment of infection, inflammation, and immune responses.
Results. Daily administration of PFD, but not GCS, was able to reduce TGFβ-enhanced viral load in lung homogenates ($p<0.05$), as measured by plaque assay. In BALF, the chemokine RANTES was reduced by both PFD and GCS, however IL-6, TNFα and KC were only reduced by PFD.
Conclusion. This study demonstrates that inhaled PFD was able to afford protection against TGFβ-enhanced viral infection and inflammation more effectively than the current standard treatment, GCS. These promising findings offer the possibility of repurposing PFD to treat patients with respiratory disease during viral exacerbations, offering protection from worse disease outcomes.

494 CXCL17 is a novel MRGPRX2 agonist: importance of cellular context

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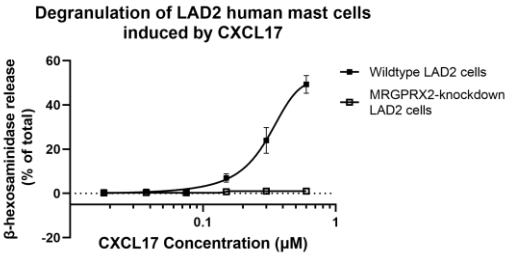
Introduction. The Mas-related G protein-coupled receptor X2 (MRGPRX2) is selectively expressed on mast cells and is activated by diverse polycationic peptides[1]. CXCL17 is a novel chemokine implicated in various inflammatory conditions but the receptor/s it acts upon remain unclear. CXCL17 has clustered regions of high cationic charge and thus we hypothesised that it may act as a novel MRGPRX2 agonist.



Aims. To determine if CXCL17 can act as a MRGPRX2 agonist.

Methods. We used the human mast cell line LAD2 that natively expresses MRGPRX2, MRGPRX2 knockdown LAD2 cells generated by CRISPR-Cas9 technology and HEK293 cell line expressing human MRGPRX2. Calcium mobilization in response to CXCL17 and other MRGPRX2 agonists was measured using fura-2. Degranulation of LAD2 cells was quantified by release of β-hexosaminidase. CXCL17 binding to MRGPRX2, and G protein-activation were determined using NanoBRET™ assays.

Results. CXCL17 triggered a concentration-dependent mobilisation of Ca^{2+} and degranulation in LAD2 cells. These effects were MRGPRX2-dependent as receptor knockdown significantly reduced CXCL17-induced Ca^{2+} mobilisation and degranulation (Fig 1). However, CXCL17 failed to trigger Ca^{2+} mobilisation and Gq activation in MRGPRX2-transfected HEK293 cells.

Discussion. We have shown for the first time that CXCL17 is an agonist at MRGPRX2 and may thus underpin some of the pro-inflammatory actions of the chemokine particularly in the skin where MRGPX2 is highly expressed. However, our results show that MRGPRX2 activation by CXCL17 is cell context specific, suggesting that other membrane components unique to the mast cell are necessary for productive receptor activation. Ongoing work aims to define this pathway which will extend our understanding of MRGPRX2 activation and its role in inflammatory disease.

[1]McNeil et al (2015) Nature 519: 237-241.

495 Adding a Dimension to Biomechanical Cues Underlying IPF

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Introduction. Idiopathic Pulmonary Fibrosis (IPF) is a progressive disease in which the lung parenchyma undergoes irreversible remodelling. Remodelling propagates from fibrotic foci, regions characterized by excess deposition of extracellular matrix (ECM) driven by activated myofibroblasts. The myofibroblast fibrogenic activity is driven both by biomechanical and biochemical cues. We have demonstrated that modulating the stiffness of the matrix microenvironment can revert the myofibroblast to a phenotype that appears 'afibrogenic.'

Aims. The present study seeks to reveal signalling pathways altered by the stiffness and dimensionality of the microenvironment in order to explain the 'afibrogenic' nature of fibroblasts cultured in the soft microenvironment.

Methods. Pulmonary fibroblasts were cultured either in a 2D stiff (conventional monolayer) or 3D soft (spheroid) microenvironment and the phenotypes engendered by these settings were characterized by a multi-omics approach, complemented with measurements of conventional fibrotic markers. To ascertain the extent to which fibroblasts in the soft microenvironment remain 'afibrogenic,' fibroblast spheroids will also be maintained for up to 28 days.

Results. Compared to fibroblasts cultured as a stiff 2D monolayer, fibroblasts cultured in the 3D soft microenvironment showed striking downregulation of actin cytoskeleton and stress fiber proteins, multiple subtypes of fibrillar collagen and connective tissue growth factor and upregulation of lipofibroblast markers such as perilipin 2. Additionally, fibroblasts in the soft, spheroid microenvironment showed altered signalling pathways, evidenced by a marked increase in the production of interleukins 6, 8 and 11, and remodelled signalling by TGF-β, one of the best characterised fibrogens, which caused notable increases in myofibroblast activation for cells in the 2D stiff microenvironment.

Discussion. This study highlights fibroblast sensitivity to the mechanical microenvironment; that acute exposure to a soft environment causes dedifferentiation from the myofibroblast to a phenotype with resistance to TGF-β-induced myofibroblast activation. Our ongoing long-term spheroid culturing will subsequently allow us to determine the persistence of the 'afibrogenic' phenotype.

496 Cell shape: a determinant of TGF-β functionality?

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Introduction. Cells are constantly exposed to isometric tension *in vivo* and have adapted the ability to retrieve guiding cues and convert mechanical stimuli into electrochemical activity, known as mechanotransduction. Changes in the microenvironment can distort the mechanical balance between the cells and their surroundings, resulting in a myriad of diseases such as Idiopathic pulmonary fibrosis (IPF). 2D culture plate and 3D spheroids are commonly used *in vitro* tools for understanding IPF. However, both systems display different microenvironmental factors such as cell-substrate interaction, cell shape, nuclear aspect ratio, cell volume, and cell spreading that could indirectly influence cell response. The feasibility to constrain cells within specific areas and shapes allows the study of cell shape in relation to existing mechanosensing pathways and their impact on fibrogenesis.
Aims. This work aims to elucidate the influence of cell aspect ratio on fibroblast fibrogenesis and whether the change of cell shape can prevent responses to TGF-β treatment.
Methods. To investigate the effects of cell shape on fibroblast fibrogenesis, cell shape was confined to distinct aspect ratios by collagen patterning. 500μm^2 collagen micropatterns were created by using deep UV (189nm) to cleave PEG moiety from PLL-g-PEG (Poly(l-lysine)-g-poly(ethylene glycol). Immunofluorescence revealed the impact of cell shape on the expression of YAP, α-SMA, and vimentin.
Results. YAP nuclear localization was observed in both cells with low and high aspect ratios. The expression of vimentin has shown to be higher in high aspect ratio cells while no significant differences were observed for α-SMA expression. Nevertheless, cable-like alpha-SMA fibres were observed only in high aspect ratio cells while low aspect ratio cells display perinuclear localization of alpha-SMA fibres.
Discussion. These observations suggest that cell shape is a key factor in influencing fibrogenesis in fibroblast cells. Thus, in addition to change in microenvironmental stiffness, fibrosis may be driven by the change of cell shapes.

497 Characterising the Contribution of Casein Kinase 1 delta (CK1δ) in the Fibrogenesis of IPF

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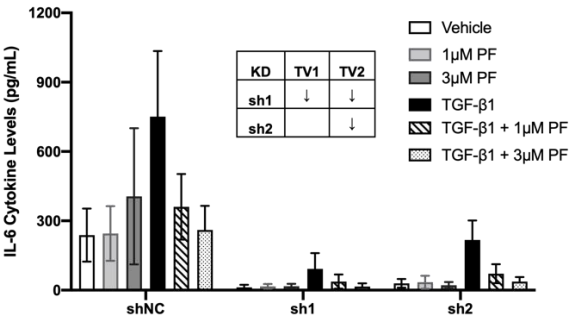
Introduction. TGF-β plays a critical role in the development and progression of the fibrotic disease Idiopathic pulmonary fibrosis (IPF). However, TGF-β targeted therapeutic strategies are not a favoured approach. Treatment of IPF is decelerated due to the limited effective pharmacological modulators. Previous work has demonstrated that the CK1δ/ε dual inhibitor PF670462 (PF) has anti-fibrotic actions *in vitro* and *in vivo* (Keenan et al., 2018).
Aims. To elucidate the contribution of CK1δ and the transcript variants (TV1-3) in fibrogenesis and to identify downstream signalling substrates with genetic studies and proteomics.



Methods. CK1δ shRNA knockdown (KD) MRC-5 lung fibroblast cells that targeted specific or all TVs were used. ELISA and western blot were used to measure key fibrotic markers of the KD cells. Global and phospho-proteomics were used to systematically investigate the proteome downstream of TGF-β1 and PF signalling and the phosphorylation of CK1δ.
Results. PF has consistently attenuated the effects of TGF-β1 simulated IL-6, IL-11, PAI-1 and CTGF cytokine levels, and α-SMA and Collagen 1A expression in MRC-5. Baseline and TGF-β1-stimulated IL-6 levels were reduced with CK1δ KD. PAI-1 and CTGF mediating through the canonical TGF-β/Smad-dependent signalling cascade remain unaffected.
Discussion. The loss-of-function approach has been useful in understanding some aspects of fibrogenesis. Elucidating the signalling mechanisms by which PF mediates its antifibrotic effects will provide valuable insights for future discovery and development of CK-targeted therapies. The emerging roles and regulation of the distinct CK1 isoforms have highlighted the need for accurate ascertainment of the physiological substrates of CK1.
[1]Keenan CR et al. (2018) Fibrosis. Front. Pharmacol. 9:738.

498 Academic student partnership in developing an online workshop to address vaccine hesitancy

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Introduction. Vaccine hesitancy is one of the top ten global health issues (1). Addressing vaccine hesitancy plays a vital role in safeguarding public health and maintaining individual wellbeing. In the context of COVID 19 pandemic, vaccination reduces the risk of serious disease, hospitalisation and death (2).
Aims. To address vaccine hesitancy and promote the benefits of vaccination, an online learning workshop was designed and delivered by the academics and student leaders in partnership to equip pharmacy and pharmaceutical science students with the necessary knowledge and skills.
Methods. An online webinar was first piloted at the University college of London and expanded to the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University in Australia, as part of a wider PharmAlliance partnership program. The webinar was transformed into an interactive online workshop jointly delivered by Monash academics and student leaders. Pre and post-workshop survey questionnaires compared participants' knowledge and beliefs regarding vaccines and vaccination and explored the actions taken to address vaccine hesitancy.
Results. The mean percentage of student participants who correctly answered the knowledge-related questions in the questionnaires improved from 89% pre-workshop to 95% post-workshop. Belief-related questions average score also increased from 83% to 88% post-workshop. 90% of students believed vaccines were safe to use pre-workshop compared with 100% post-workshop. Pre-workshop, 37% of students believed they would engage with a vaccine hesitant stranger compared with 75% post-workshop.
Discussion. The online workshop to address vaccine hesitancy was designed and delivered by the academics and students in partnership. Students took charge of promotion and recruitment, surveys and workshop presentation including roleplay and small group discussions. Academics ensured the content accuracy, mentored student presenters and moderated discussions. Post-workshop, participants felt better prepared and confident to address vaccine hesitancy with family, friends and communities.
(1) Dube E et al (2021) Annu Rev Public Health. 42:175–91. (2) Moghadas SM et al (2021) Clin Infect Dis. 73: 2257-2264.

499 A faculty approach to unify the Honours program in Pharmaceutical Science

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Introduction. The Honours Program at the Faculty of Pharmacy and Pharmaceutical Sciences provides comprehensive research training to undergraduate students enrolled in the B. PharmSci (Hons) or B.PharmSci (Advanced Honours) degrees. Students undertaking Honours can enrol through 5 distinct 'themes' established at the Faculty however historically, the Honours program has been largely theme-dependant without consistency across the themes.
Aims. To unify the Honours Program at the Faculty of Pharmacy and Pharmaceutical Sciences, at Monash University.
Methods. 'Town hall' meetings were held with staff to receive feedback regarding the proposed course structure. Honours theme leads were surveyed to identify core knowledge/skills required to successfully complete honours in the faculty's themes. Based on survey responses, 9 topics/skill areas were identified and formed the basis for the development of coursework modules in consultation with key academic staff. Modules were delivered through the learning management system (LMS) with some modules supported with hands-on activities. The assessment of the research component was consolidated such that all themes had a shared assessment procedure, supported by newly-designed rubrics. The Research Unit was also supported by an LMS site.
Results. Nine coursework modules were developed using an evidence-based approach to ensure modules were user-friendly, flexible and self-regulated with a focus on learner engagement. As part of the Honours coursework component, all students completed the 'research skills' module and selected 2 additional modules to complete in consultation with their supervisor. Notably, students working across themes could select modules that would satisfy the coursework requirements of each theme. In the research component, students completed common assessments with common weightings, including a manuscript, final presentation and oral viva. The redesigned Honours' Program was delivered for the first time in 2021. Student evaluations revealed >90% of students were satisfied with the coursework and research units. Suggestions for future improvements included additional feedback and timetabling.
Discussion. The Honours program in Pharmaceutical Science was redesigned and redeveloped to have a unified approach; the revised course will allow for meaningful comparison of students with regards to knowledge and skill development. Feedback obtained from staff and students will be used to further refine the course.

500 Student perceptions of pharmacology laboratory experiences: computer simulation vs in-person "wet" lab

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Introduction. There has been a shift in recent years in higher education towards the use of online and virtual laboratory experience in place of in-person "wet" laboratory classes that was accelerated by the COVID-19 pandemic. However, the students' perceived value of these type of classes in terms of development of learning outcomes and professional skills is not well known in a pharmacology class setting.
Aims. The aim of the current study was to determine whether students perceive computer simulations to be equivalent, better, or inferior to wet labs in terms of development of a student's understanding of learning outcomes and professional skills following the session.
Methods. 385 students from the PHA2022 Drugs and Society unit at Monash University undertook a computer simulation in one week followed by a "hands-on" wet lab the next week. Following each class, the students were invited to complete a survey via Qualtrics which asked to what extent they agreed that they had developed a list of 11 professional skills and 4 learning outcomes on a 4-point Likert scale (Strongly Disagree – Strongly Agree). 74 and 44 students (19-11% response rate) participated in the surveys following the computer simulation class and "hands-on" wet lab, respectively.
Results. The current study found that there was a greater proportion of students who felt they had a stronger development of their professional skills (e.g. communication & teamwork) following the "hands—on" wet lab compared to the computer simulation. There was also a noticeable increase in the number of students who felt they had a stronger understanding of the main learning outcomes following the wet lab compared to the computer simulation.
Discussion. The findings of this study highlight that the perceived gains of a wet lab vs a computer simulation class extend beyond just discipline specific learning outcomes. The students also seem to perceive and value a greater development in important professional and communication skills following the wet lab, which is highly relevant to their interactions with their peers and colleagues.

501 Using clinical case studies to build work relevant skills

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Introduction. Integration of work relevant skills into teaching pharmacology of science courses is a required and important part of developing future professionals. These teaching approaches provide graduates engagement in authentic work contexts where individual professionals practice and to participate and engage with interactions that constitute to the "clinical practice" environment.
Aims. The aim of this study was to broaden student skill set, including professional communication, teamwork, leadership and clinical practice decision making using clinical based case studies.
Methods. Students work in team of 9-10 members, with a group leader being appointed per team. Two specific tasks, which comprised of self-learning and building of teamwork and partnership are required to be completed per set time. For self-learning, each member first solved an allocated clinical issue, presented as a case study. For team learning, each team get together to discuss, negotiate and agree on the solution, then presented their decision and outcome to other teams by mean of a power point presentation. The measurement and assessment of the implemented class activity were facilitated by peer assessment, in which the presented outcome by each team was judged and provided a score by other teams' members. The role of team leader was to facilitate their team learning and participation, as well as reporting peer assessment and score to Course coordinator.
Results and Discussion. A total of 72 DVM students were enrolled in the Pharmacology course in 2022, who participated in the learning activity. To run the implemented practice-based class activity successfully, key resources required include clinical based case studies with a problem/issue to be addressed and materials such as Google doc.; MyUni online Course; online approach/method for documentation, collating of team discussion, and reporting of assessment and power point app for preparation of presentation. In addition, F2F attendance and active engaging, contributing to team decision were also required by all members. Overall, students' performance and confidence appeared improved greatly over time, specifically in their written, oral communication and presentation skills. The implemented class activity was also found to greatly facilitate student engagement and participation in their learning.
Conclusion. In summary, clinical case studies were successfully utilised in a pharmacology course to develop teamwork skills, problem solving, and decision making in a team-based setting and to increase active engagement, participation.

502 Student perspectives on the effects of cognitive enhancers and attitudes towards use

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Introduction. Use of cognitive enhancers (CEs), also known as study or smart drugs, is escalating world-wide (Sharif *et al.* 2021). While personal use has been extensively investigated, little is known about users' knowledge of the drugs' actions and adverse effects (AEs). Similarly, there is minimal insight into students' attitudes towards these drugs.
Aims. To use student focus groups to provide the foundation for the development of a questionnaire to investigate their peers' perceptions of the actions and adverse effects of CEs, as well as attitudes towards their use.
Methods. Students enrolled in the University of New England (UNE) Pharmacy program were recruited in 2021 and 2022 for 2 sets of focus groups. The first session engaged participants in a discussion about their peers' potential knowledge and understanding of CE action (UNE Human Ethics Approval HE21-113). Thematic analysis of session transcripts was used to create a draft questionnaire, which was given to the second focus groups to critique. Thematic analysis of the resulting transcripts informed the development of the final questionnaire.
Results. A total of thirteen students (6 women, 7 men), aged 22-54 years, participated in this study. Group members believed that few of their peers would understand how CEs worked and would be more concerned with whether they worked. Uniformly, young men were perceived as being much more likely to try these drugs, while most participants thought that AEs would have to be severe and immediate to discourage use. Cognitive enhancement was generally seen as acceptable, and furthermore, that use of such drugs did not constitute academic misconduct.
Discussion. Resembling the disinterest in the action of recreational drugs by those who use them, students lack understanding of how CEs work. Acceptance of the use of these drugs while studying, however, is in stark contrast to the perception of practicing pharmacists (Ram *et al*. 2020). This project builds on the existing literature to propose a tool to explore the gaps in the knowledge of the action and perception of the use of CEs at Australian universities.

Sharif S *et al*. (2021) Brain Sci 11(3):355
Ram S *et al.* (2020) PLoS ONE 15(11):e0241968

503 How do technology-enhanced learning strategies affect biomedical and healthcare students' engagement?

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Technology-enhanced learning strategies have proliferated since the COVID-19 pandemic. However, a review of literature has identified that many technologies used for remote emergency education were crisis management tools lacking a focus on student engagement and any pedagogical underpinning. Using constructivism and connectivism frameworks to understand student engagement, this study aims to identify engaging technologies used in healthcare and biomedical student education. Student engagement with technologies was measured through a hybrid scale, customised from the published Student Engagement in e-Learning Environment Scale (SELES) and Distance Education Learning Environment Scale (DELES). Interviews with students and educators, were utilised to effectively triangulate the findings. Quantitative data were analysed using SPSS for statistical interpretations while qualitative outcomes were thematically analysed.

Preliminary findings indicate that whilst students are engaged in online learning using various technological tools, they prefer to experience face-to-face teaching supplemented by effective technologies. Both students and educators identify the need for learning to be focused on developing skills and graduate qualities, as opposed to traditional content knowledge-focused teaching. These findings are consistent with theories of constructivism where knowledge is constructed through experiences and participation in valued practices, and connectivism, which emphasises the importance of technologies and networks in the creation of knowledge. Outcomes of this study will help diagnose areas for improvement and sustainability in the digital learning space post COVID-19.

Brown T et al (2022) Am J Distance Educ 1-18 DOI: 10.1080/08923647.2022.2065147
Elshami et al (2022) Nurse Educ Today 110, 105261
Karimian Z et al (2022) Educ Inf Technol 27(3):3299-3320
Mattar J (2018) Rev Iberoam educ 21:201-217

504 Going digital – students' perceptions of electronic laboratory notebooks (ELNs)

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Introduction. Laboratory notebooks are an essential part of any experimental investigation. ELNs offer advantages over traditional paper-based notebooks, including flexibility in content types, data security, auditing and collaboration[1]. The introduction of ELNs into undergraduate classrooms needs to consider the barriers and facilitators from the perspective of the students.
Aims. To determine the facilitators and barriers of ELNs from the perspective of student users.
Methods. Undergraduate pharmaceutical sciences students used an ELN for the 5 laboratory sessions in a Year 3 biotechnology unit. All 95 enrolled students were invited to complete a survey of Likert-type and open-ended questions to ascertain their experiences using the ELN at the end of the semester. A total of 61% of the class completed the survey.
Results. Accessibility appeared as a frequent theme in the open-ended question regarding what students liked most about the ELN. However, it is noted that this requires access to an appropriate device, and while many students found the functionality of the ELN easy to navigate, others expressed technological and system-related difficulties that detracted from the experience. Students had access to a limited number of iPads in the classes and the influence of this access was also captured in the survey responses.
Discussion. Access to suitable hardware is a significant barrier to the uptake and successful implementation of ELNs in laboratory classes (Kanza *et al.*, 2017). Furthermore, student familiarity with the functionality of the software is essential to ensure they can access the full benefits of the system to maximise collaboration with both staff and peers.

[1]Higgins et al (2022) Nat Protoc 17:179-189
[2]Kanza S et al (2017) J Cheminform 9:31

505 The patient perspective: understanding the breast cancer medication management process

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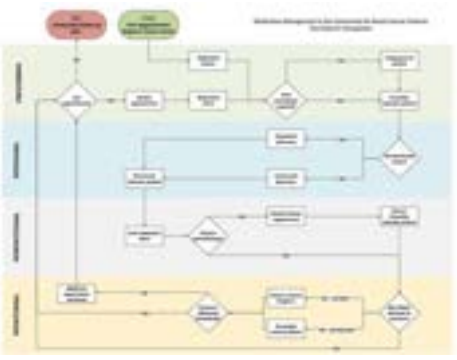
Introduction. People with breast cancer undergo multiple transitions of care. Service fragmentation creates barriers to coordinated, integrated care. Medication management during transitions of care presents multiple challenges for people with breast cancer. Few studies examine patients' perspectives of medication management at transitions of care.
Aims. To: A) Develop a process map to elucidate medication management for patients with breast cancer during transitions of care; B) Understand patients' perspectives on the relevance of the co-designed process map.
Methods. Single-site case study design comprised two sequential phases: (1) process mapping of medication management generated from literature and insights from a medical oncologist and breast cancer nurse and (2) semi-structured interviews (n=10) with patients with breast cancer to ascertain the usefulness of the process map to explore medication management during transitions of care.
Results. The process map in the figure described the phases of prescribing, dispensing, administering and monitoring. The process map provided a realistic outline of the process the participants experienced. They acknowledged the role of different team members, including pharmacists and nurses, though many perceived the siloing of health professionals as a barrier. However, it was recognised that the medical oncologist played a dominant role in driving this process. Patients also reported a high burden of managing of information from multiple sources.



Discussion. The oncologist was the driver of this process, highlighted by the relatively minor role played by others created a high burden on oncologists and patients. They reported inconsistencies in the quality of communication and education they received. Interviews with key professionals and support persons will help identify possible modes of change.

506 Examining the Aboriginal and Torres Strait Islander Cultural Safety content in Australian Pharmacy Schools: - a Qualitative study

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Introduction. Historically there was very little awareness of Aboriginal and/or Torres Strait Islander people's histories and cultures nor understanding of colonisation and the resultant health disparity. The APC requirements for pharmacy degree accreditation state that program planning, design, implementation, evaluation, review, and quality improvement processes are to be carried out in a systematic and inclusive manner. These requirements specifically mandate that Aboriginal and/or Torres Strait Islander people be included amongst consulted stakeholders when a degree program is being proposed or reviewed for accreditation
Aims. To explore academics' views on Aboriginal and/or Torres Strait Islander Health and Cultural Safety content in pharmacy school curricula to inform recommendations for future curricula.
Methods. All 18 Australian pharmacy schools were contacted, and interviews were conducted with consenting Heads of school, or their delegate/s. Audio recordings of interviews were transcribed verbatim. Transcripts were thematically analysed and mapped to the Aboriginal and Torres Strait Islander Health Curriculum Framework.
Results. All 18 schools consented to participate and a total of 22 interviews were conducted. Many Interviewees expressed that the current content regarding Aboriginal health and cultural safety/competence was lacking in many schools and cited barriers that have led to lack of development. Other schools that had undergone recent curriculum reform however, had purposefully designed content scaffolded across the degree. Interviewees expressed multiple ideas for how new curricula could embed cultural safety/competence and ideas for sustainable change moving forward.
Discussion. Whilst the Aboriginal and Torres Strait Islander Health Curriculum Framework was introduced in 2014, its dissemination into pharmacy curricula overall appears to be poor. Despite this, the Australia Pharmacy Council guidelines are well known to most educators. It is apparent that pharmacy schools are at different stages in their development of Aboriginal and Torres Strait Islander Health curriculum design and implementation and future resources should be developed and made available.

507 Clinical impact of Antibiograms as an intervention to optimise antimicrobial prescribing and patient health outcomes – a Systematic Review

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Introduction. Overuse and misuse of antibiotics contributes significantly to the development of antibiotic resistance. Sub-optimal choice of antimicrobials can lead to poor infection related outcomes. Antimicrobial stewardship (AMS) guidelines advocate for the use of antibiograms as a tool to guide empirical antibiotic prescribing and inform local treatment guidelines.
Aims. To evaluate the clinical impact of antibiograms as an intervention to optimise antimicrobial prescribing and patient health outcomes.
Methods. Systematic review. Embase, PubMed, CINAHL and IPA databases were searched from inception until September 2022, to identify studies of antibiogram related interventions for optimising antimicrobial use and/ or patient outcomes in all health care settings. Two reviewers independently screened title and abstracts and full articles. Risk of bias was assessed using NIH Quality assessment tool depending on the nature of the study.
Results. Thirty-seven studies met inclusion criteria and were included for review. Majority of studies were conducted in the United States (68%) and in hospital settings (73%). All interventions were multifaceted and in 24 (65%) studies, antibiograms could be considered as an integral component of the interventions with most reported to be specific to the individual facility. Six studies were considered to have poor quality, 26 as fair quality and five were considered as good quality. There was a positive impact on antibiotic consumption trends (8 studies) and appropriateness of prescribing (8 studies) with limited evidence for some improvement in infection related outcomes, mortality, resistance profiles and costs associated with antibiotic use. Insufficient data prevented meta-analysis of any outcomes due to lack of control groups.
Discussion. Clinical use of antibiograms to inform treatment recommendations may be an effective AMS strategy to improve empirical antibiotic prescribing however further good quality studies with rigorous study designs are recommended to evaluate its effectiveness in less studied health care settings such as aged care.

508 Exploring current teamwork skill aptitudes of first year undergraduate students studying Pharmaceutical Science

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Introduction. Effective communication and collaboration underpin effective teamwork skills and are essential to the pharmaceutical science workforce (Cooper et al., 2022). Teamwork does not occur as a consequence of putting people together. Students require guidance, teaching, and mentorship to develop these skills. A survey instrument tool (Baseline Teamwork Assessment Tool) was developed at the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University in 2021 to measure baseline teamwork skills in students. The tool was administered to first year Pharmaceutical Science students in 2021 (onshore: n=192; offshore: n=42) at the beginning of the academic year.
Aims. To characterise the baseline teamwork aptitudes of 1st year undergraduate Pharmaceutical Science students using free text survey responses to a 3-part teamwork scenario.
Methods. Existing literature on common teamwork attributes in students was reviewed using PubMed & CINAHL search engines. Backward citation searching was used to shortlist 7 articles (of ~293) published in 1999-2015 for further analysis. From these, 11 deductive themes were identified on common strategies utilised by students to navigate common teamwork challenges. Subsequently, inductive thematic analysis was adopted in the coding of a subset of the available data (n=30). This resulted in the identification of 3 additional themes to a total of 16 themes.
Results. The theme of "Understanding" was common throughout student responses, indicating their intentions to evaluate the problem to find an appropriate solution, and their care and empathy for team members. "Awareness" was also common, as students wanted to clarify the situation. "Compromise" demonstrates students' common belief that individual behaviour can be changed with the right environment and support. "Correcting behaviour" was seen often, as students demonstrated the intention to inform peers of their own beliefs about how teamwork should be performed and the responsibilities of each team member.
Discussion. This study identifies common strategies used by students to navigate common teamwork challenges and also highlights areas student lack the knowledge and skills overcome barriers to effective teamwork. Findings from this study will be used to develop resources and instructions to support the development and cultivation of teamwork skills in undergraduate students.

509 Pharmacy students' health literacy knowledge: a qualitative exploratory study

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Introduction. Health literacy – a person's ability to access, understand, appraise and use health information – is an important concept which influences individual health outcomes. Pharmacists are ideally positioned to provide additional support to people with inadequate health literacy, and there have been worldwide recommendations to integrate health literacy education into tertiary-level health professions curricula. However, the extent of health literacy education in Australian pharmacy degrees is unknown.
Aims. This research explored pharmacy students' health literacy knowledge.
Methods. A qualitative, web-based questionnaire was disseminated to pharmacy students enrolled in an Australian university. Questions explored students' fundamental health literacy knowledge: the definition, signs and consequences of inadequate health literacy, and actions that pharmacists can take to assist patients to counteract this. Responses were inductively themed and reported descriptively.
Results. Of the 31 pharmacy students who participated, overall health literacy knowledge was low. Students frequently identified understanding and knowledge as components of health literacy; however, most students did not identify that health information access, appraisal and use were additional components. Signs and consequences of inadequate health literacy were better understood, but suggested actions to help patients address inadequate health literacy were vague and potentially ineffective. Strategies to tailor health information to patients' health literacy level and evaluate patient understanding were also poorly understood.
Discussion. This research uncovered major gaps in pharmacy students' health literacy knowledge, specifically when defining health literacy and ways to help patients with inadequate health literacy. Graduating pharmacists who do not possess a solid understanding of fundamental health literacy knowledge will struggle to help patients overcome health literacy-related challenges in practise. Further exploration of health literacy education curricula and techniques is urgently recommended to improve students' understanding of health literacy.

510 Non-*DPYD* gene variants associated with severe fluoropyrimidine toxicity

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Introduction. Loss of function variants in the *DPYD* gene, which encodes dihydropyrimidine dehydrogenase (DPD), have high specificity but very poor sensitivity for prediction of the risk of severe to life-threatening fluoropyrimidine toxicity. We undertook a case-control study of patients treated with fluoropyrimidine monotherapy (ACTRN12615000586516) and phenotyped the cohort for DPD activity using both a thymine challenge test (Helsby et al, 2020) and endogenous uracil (Burns et al, 2021). Those studies suggested that many of the severe to life-threatening toxicity cases did not have low DPD enzyme activity, with sensitivities of 57% and 29% for the thymine and uracil tests, respectively, and 14% for genotyping of the clinically relevant *DPYD* variants (Burns et al, 2021).
Aims. To undertake an exploratory *post hoc* analysis of associations between risk of severe fluoropyrimidine toxicity and single nucleotide polymorphisms (SNP) in a) drug transporters and b) mediators of inflammatory responses.
Methods. Patient DNA samples were analysed for variants in 5 drug transporter and 16 inflammatory genes by MassARRAY. Associations between groups (≥G3 toxicity cases; ≤G2 non-cases) and genotypes (categorical data) were assessed using Fisher's Exact Tests in IBM SPSS Statistics 26.
Results. SNP in *ABCC11* (rs16945916, p=0.044), *SLC23A2* (rs4987219, p=0.033) and *OPRM1* (rs1799971, p=0.004) appeared to associate with severe toxicity (unadjusted p-values) in the cohort (N=35). In the subset of the patients recruited prospectively (N=29) the relative risk of being a ≥G3 toxicity case was 2.62 (95%CI = 1.52 to 4.53) for *OPRM1* rs1799971 homozygous wild-type (AA) carriers; 3.33 (95%CI = 0.95 to 11.66) for *ABCC11* rs16945916 variant allele carriers (TC+CC); and 0.75 (95%CI = 0.50 to 1.12) for carriers of *SLC23A2* rs4987219 wild-type alleles (CC+CG).
Discussion. These data should be interpreted with caution due to the small and underpowered nature of this *post hoc* analysis. We will assess whether the association of these SNP with severe fluoropyrimidine toxicity can be replicated in an independent prospective cohort (ACTRN12617001109392; N=166).
Helsby, NA, et al (2020). Br J Clin Pharmacol 86(1): 155-164.
Burns, KE, et al (2021). Cancer Chemother Pharmacol 87(5): 711–716.

511 Pharmacogenomic implications of polypharmacy in hospitalised patients taking antipsychotic medications

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Introduction. Polypharmacy and inter-individual genetic variations can be barriers in receiving effective pharmacotherapy for mental illness. Translating drug-gene associations into clinical practice through pre-emptive pharmacogenomic testing could enhance treatment outcomes by reducing adverse effects and increasing efficacy.
Aims. To investigate the pharmacogenomic implications of polypharmacy in patients taking at least one antipsychotic medication before hospitalisation.
Methods. This retrospective observational study utilised existing inpatient data from three South Australian public hospitals between 1 January to 31 December 2019. Patients' discharge medications were categorised as inhibitors, inducers, and substrates according to PharmGKB for CYP2D6, CYP3A4, CYP3A5 and CYP2C19. Drug-drug interactions were checked using MIMS, Stockleys and Medscape.
Results. There were significant differences in the number of CYP2D6, CYP3A4/5 and CYP2C19 substrates between acute and non-acute patient groups (p=0.0012). Olanzapine and quetiapine were the most frequently prescribed second generation antipsychotic medications to the acute (33.2%) and non-acute (36.3%) patients respectively. The prevalence and severity of drug-drug interactions between acute and non-acute patient groups varied greatly. For acute patients, the drugs identified as most prescribed were substrates of their respective enzyme, and thus as metabolism of other drugs is not likely be affected, additive effects are most likely to be seen. In comparison, combinations of substrates and inhibitors and/or inducers of their respective enzyme were more likely to be seen upon discharge in non-acute patients, and therefore interactions involving metabolism of other medications are significant.
Discussion. Our study identified that non-acute patients are at a higher risk of being discharged with potentially avoidable interactions that effect the efficacy of treatment for mental illness. Incorporating pharmacogenomic guided prescribing would lead to better patient outcomes in this cohort.

512 Dihydropyrimidine dehydrogenase (DPD) phenotyping methodologies: A literature review

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Introduction. Dihydropyrimidine dehydrogenase (DPD) is a key enzyme in the metabolism of fluoropyrimidines (FP), such as 5-fluorouracil (5FU) and capecitabine. DPD accounts for >80% of 5FU catabolism and deficiency is associated with 5FU induced toxicity. Therefore, pre-emptive DPD activity testing has been recommended prior to commencing FP-based chemotherapy. Several methodologies to measure or predict DPD activity exist.
Aims. To conduct a literature review to identify and evaluate current DPD phenotyping methodologies.
Methods. Literature searches were conducted in Medline, Embase and Scopus databases. Only manuscripts in the English language published between 2017-2022 were included. 232 manuscripts were identified, and 13 were included in the literature review.
Results. There were four main DPD phenotyping methodologies identified in the literature: (i) DPD enzyme activity measured directly in peripheral blood mononuclear cells (PBMCs); (ii) DPD enzyme activity predicted by measuring endogenous pre-treatment plasma uracil (U) and its metabolite dihydrouracil (DHU), and DHU/U ratio; (iii) Administration of oral 2-^{13}C-uracil (stable isotope of U), followed by measurement of either exhaled $^{13}CO_2$, or U and DHU plasma concentrations; and (iv) 5FU degradation rate determined in intact PBMCs used to predict the risk of FP toxicity.
Discussion. The identified DPD phenotyping methodologies are each associated with different advantages and disadvantages. Factors that require consideration for methodology selection include sensitivity, sample stability, practicality, time-efficiency, cost, patient compliance and requirement of specialised equipment. Before DPD phenotype-guided dosing of FP-based chemotherapy can be utilised in clinical practice, further prospective studies are required to validate threshold values for classification of DPD deficiency.

513 Investigating How Polypharmacy and Deprescription Alter the Hepatic Proteome in Aged Mice

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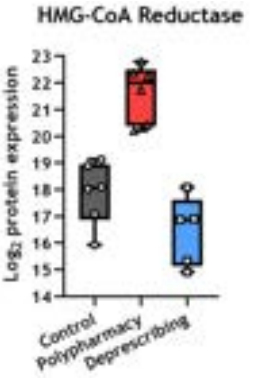

Introduction. Polypharmacy (concurrent use of ≥5 medications) with increasing Drug Burden Index (DBI; total exposure to anticholinergic and sedative medications) is common in older Australians and is associated with adverse geriatric outcomes such as frailty and cognitive impairment. Deprescribing (withdrawing) may alleviate some of these outcomes. The liver has roles in pharmacokinetics, pharmacodynamics, and ageing. Molecular effects of polypharmacy and deprescribing on aged liver are unknown.
Aims. To investigate the effect of polypharmacy and deprescription on the hepatic proteome in aged mice.
Methods. Healthy C57BL/6J mice at 12 months of age, received either (i) control, (ii) chronic polypharmacy treatment (14-month treatment), or (iii) chronic polypharmacy followed by deprescription (9-month treatment, 5-month deprescription) (*n*=6/group). Polypharmacy regimen (therapeutic doses of simvastatin, metoprolol, citalopram, oxycodone, oxybutynin) studied has a high DBI score (>1.0). Liver samples were harvested at age 26 months, and mass spectrometry-based quantitative proteomics was performed by data-independent acquisition (DIA) using a Q-Exactive (Hfx) orbitrap mass spectrometer. Differential expression, pathway, and network analyses were applied.
Results. Preliminary results suggest compared to control, polypharmacy causes differential expression of 50 proteins, however following deprescription (compared to polypharmacy) 61 proteins change (Student t-test; fold-change threshold: ±1.5; FDR < 0.05). Chronic polypharmacy alters protein expression of drug metabolism, cholesterol biosynthesis, and amino acid metabolism, among others, for which deprescription has some reversal effects; for example, HMG-CoA reductase (protein target of simvastatin, see figure).
Discussion. Preliminary results suggest chronic polypharmacy altered expression of hepatic proteins and some changes were reversible with deprescription. Future work will explore monotherapy treatments to understand the interactions and mechanisms of changes seen with polypharmacy.

514 Outcomes and popPKs of antiviral drugs used in COVID-19 patients: A review

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Objective. The aim of this study was to provide a summary on human clinical studies that investigated outcomes and population pharmacokinetics of anti-viral drugs plus other therapy used to treat COVID-19 positive patients.
Methods. A systematic review was conducted in PubMed, EMBASE, and the Cochrane Library from January 2020 to October 2022. Human clinical studies, published in English were selected. Selection criteria: studies investigated population pharmacokinetics, studies investigated antiviral drugs or other therapy and studies examined COVID-19 patients.
Results and Discussion. Four clinical studies were identified. The investigated drugs were lopinavir/ritonavir, darunavir, favipiravir and bamlanivimab/etesevimab. Key findings of the clinical trials are summarised in Table 1.
Studies utilised in silico approaches, stimulation for dosage and PKs prediction and/or investigated other repurposing drugs other than antiviral drugs will also be discussed. Additionally, three RCTs reported negative outcomes will also be discussed briefly.
Conclusion. In summary, the identified clinical studies provided insightful suggestion on dosage regimen, pharmacokinetics, and toxicity of current therapies used to treat COVID-19 positive patients.

Study ID	Sample size	Drugs	Key summary	Common covariates
Alvarez et al 2020[1]	13 hospitalized Covid-19 positive patients 4F; 9M 65 (+/-16)-year-old	Lopinavir/ ritonavir	Aim: popPK model development Regimen: lopinavir/ritonavir 400/100 mg b.i.d. Finding: i) 400/100 mg b.i.d. showed a high variability in median concentration of 20 and 30 mg/L (Cmin/Cmax) (90% prediction interval = 1-100 mg/L) ii) A dose of 400 mg b.i.d. resulted in 40% patients < MEC iii) A dose of 1200 mg b.i.d. resulted in 22% patients <MEC	Age Sex, Body weight Liver and/or Kidney status Disease status
Chigutsa et al 2021[2]	2970 mild moderate Covid-19 positive patients	Bamlanivimab Etesevimab	Aim: Investigated antibody drug popPK Regimen: bamlanivimab/etesevimab 700/1,400 mg iv Finding: i) 700/1,400 mg resulted in optimal reduction in viral load ii) Early treatment most benefit patients	
Cojutti et al 2020[3]	30 admitted Covid-19 positive patients	Darunavir	Aim: Examined drug popPK Finding: i) Drug clearance is reduced in Covid-19 positive patients ii) Monitor drug-drug interactions, dosage adjustment	
Irie et al 2021[4]	39 Covid-19 positive patients 8F; 31M 27-89-year-old	Favipiravir	Aim: Investigated drug popPK using MS Regimen:1600 mg/600 mg b.i.d. Finding: i) 1600 mg/600 mg b.i.d (1st/2nd dose) insufficient for target 50% EC (9.7 µg/mL). ii) A higher dosage required for patients with larger BSA and/or invasive mechanical ventilation iii) Monitor toxicity with higher dose	

Table 1: Key findings from identified human clinical studies

515 Understanding the use of psychotropic medication in autistic young people in Western Australia

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Background: Significant variability in the prevalence use of psychotropic medication is reported in international cohorts of young autistic individuals. Apart from Risperidone, approved by the Therapeutic Goods Administration to manage challenging behaviours (e.g., aggression and injuries to self or others), the rationale for other psychotropic medications prescribed to autistic young individuals is misunderstood and remains under-studied in Australia.
Aim/Method: To understand the magnitude, types and indication of psychotropic medication use in autistic children and adolescents in Western Australia. We analysed de-identified data from 239 autistic individuals (≤ 21 years) who participated in the Western Australian Biological Registry (WAABR) between 2011-4.
Results: One quarter (n=66, 28%) of young autistic people reported current or previous use of psychotropic medications. Most (n=46, 70%) of those medicated were under 12 years of age; half (n=33) 6-12 years and a fifth (n=13) under 6 years. The most used medications were stimulants, antiepileptics, antidepressants, sedatives, and antipsychotics. The reported medications were mainly to manage ADHD, challenging behaviours, seizures, insomnia, undefined anxiety, depression, and mood instability.
Discussion/conclusion: Whilst most autistic young people in the WAABR did not use psychotropic medications, over a quarter were prescribed medications, primarily stimulants, to manage symptoms of ADHD. Risperidone was used to help manage challenging behaviours. Although the prevalence of psychotropic medication use in young autistic individuals appears lower than in other developed countries, medications should be carefully monitored due to limited understanding of their effectiveness in managing the atypical presentation of co-occurring disorders in young autistic individuals.

516 Prescribing patterns in very elderly inpatients in Christchurch, New Zealand

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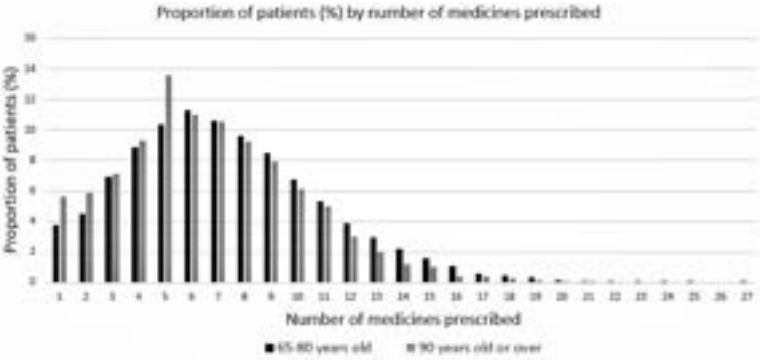

Introduction. Older age is associated with polypharmacy and adverse effects of medicines. The pattern of medicines use in very old age patients is not well understood.
Aim. To compare medicines prescribed to inpatients 90 years old or over with medicines prescribed to 65-80 year olds.
Methods. A retrospective cohort study of medicines prescribed for hospital inpatients in Christchurch New Zealand. Prescribing data for all hospital inpatients in the year 2021 were extracted from the prescribing system. Number of regular medicines active at discharge for each age group were compared using t-tests and rates of prescribing different medicines were compared using relative risk. Medicines prescribed 'as needed' were excluded.
Results. 11,422 patients (1535 patients aged 90 or over and 9887 patients aged 65-80) were included in the study. Patients aged 90 or over were prescribed fewer medicines overall (6.6 vs 7.3, P <0.001). The rate of polypharmacy (five to nine medicines) was similar (50.4% vs 52.4%, P = 0.139), and hyperpolypharmacy (ten or more medicines) was less common in the very old (19.7% vs 25.5%, P = 0.003). Patients aged 90 or over were more likely to be prescribed nighttime sedatives (RR 2.1), haloperidol (RR 3.6) anticoagulants (RR 1.2) and furosemide (RR 2.4), and less likely to be prescribed antihypertensives (RR 0.56), statins (RR 0.56), tramadol (RR 0.25) and non-steroidal anti-inflammatory drugs (RR 0.09). Proton pump inhibitor use was similar in both groups (45.7% and 44.4% respectively).
Discussion. Polypharmacy is common in very elderly patients with uncertain benefit. Night time sedation and haloperidol use was frequent in those aged 90 or over despite the high falls risk in this population.

517 Deprescribing heart failure medications in older people: a systematic review and meta-analysis.

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Introduction. Optimisation of heart failure (HF) medications in frail older people requires a complex balance of the benefits and risks of medications with disease progression and co-morbidities. The safety of deprescribing is unclear.
Aims. To determine the feasibility and safety of reducing or ceasing HF medications in older people and evaluate these outcomes by frailty status.
Methods. A systematic search of electronic databases (CENTRAL, MEDLINE, Embase, Ageline, CINAHL, IPA, PyschInfo) and registries was conducted according to PRISMA 2020 checklist. Eligible studies included randomised controlled trials (RCTs) and observational studies of people aged $\geq$50 years, diagnosed with HF and reported ceased or reduced dose of HF medications. Two reviewers independently screened all studies, validated extracted data, and assessed risk of bias (RoB2, ROBINS-I) and certainty of evidence (GRADE). If heterogeneity (Higgins I^2) was not substantial (I^2<60%), a meta-analysis was conducted on the proportion of participants whose HF medications were ceased or dose-reduced, not re-started; mortality, hospitalisation; adverse drug withdrawal effects (ADWE); and frailty status.
Results. Five RCTs (480 participants) and 22 observational studies (412,988 participants) across six drug classes were included. No study reported frailty status. Only three studies reported low risk of bias. RCTs reported successful reduced dose of an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin-renin blocker (ARB) in 64% of participants; and successful cessation of digoxin and diuretics in 52% and 53% respectively. Of these participants in the intervention group, 100%, 100 % and 76% did not re-start the deprescribed medication within 12-26 weeks. Moderate certainty showed decreased mortality from reduced dose of an ACEI/ARB (n=56) (Risk Ratio [RR] 0.64, 95%CI 0.30-1.64). There was high certainty that ceasing digoxin (n=178) reduced mortality (RR 0.30, 95%CI 0.03-2.83) but increased the risk of hospitalisation (RR 5.48, 95%CI 1.26-23.80). Overall, the most frequently reported ADWE was worsening HF and occurred in 20%-75% of participants within 5-232 weeks of deprescribing.
Discussion. Over 50% of older people with HF could cease digoxin or reduce the dose of an ACEI/ARB, but this was associated with increased risk of hospitalisation and worsening HF. Evidence is needed to support deprescribing in HF.

518 Prescribing of antiviral agents for COVID-19 in a large tertiary hospital

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Introduction. Statewide guidelines are available in South Australia to ensure the quality use of antiviral agents for COVID-19. However, protocol adherence by hospital prescribers is unclear. Aims. To determine if inpatient and outpatient antiviral prescribing and management are in accordance with South Australian guidelines. Methods. The relevant information was collected from the Sunrise Electronic Medical Record (Allscripts, Chicago, IL, USA) between 2/8/22 and 20/8/22. The appropriateness of prescribing of COVID-19 antiviral treatment was determined based on the severity of infection, renal function and potential drug-drug interactions, identified using the Liverpool COVID-19 drug-interaction checker. Results. We reviewed 40 inpatients and 9 outpatients over a two-and-a-half-week period. Prescribed agents included remdesivir (n=23, 47%), nirmatrelvir and ritonavir (n=19, 39%) and molnupiravir (n=7, 14%). Most patients had mild disease (n=33, 67%), with a smaller proportion having moderate (n=12, 25%) or severe disease (n=4, 8%). Overall, 41% of prescribing was not in accordance with South Australian guidelines. The most common reasons for non-adherence were avoidance of nirmatrelvir and ritonavir due to drug-drug interactions that were relative contraindications (n=7, 14%) and incorrect dosing of nirmatrelvir and ritonavir based on renal function (n=6, 12%). Conclusion. The appropriate management of drug-drug interactions may increase the use of nirmatrelvir and ritonavir which is less expensive than remdesivir and more effective than molnupiravir. The dose of nirmatrelvir and ritonavir often need to be adjusted based on renal function which may be overlooked by prescribers. Multidisciplinary antiviral stewardship rounds may present an opportunity to improve the quality use of antivirals for COVID-19 in the hospital setting.

519 Trends in the utilisation of antiepileptic drugs following ischemic stroke.

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Introduction. Post-stroke epilepsy is an important complication which may increase the risk of recurrent stroke and mortality. However, there are no clinical guidelines on the appropriate use of antiepileptic drugs (AED) in post-stroke cohorts.
Aims. This study aimed to investigate trends in the utilisation of AED and examine predictors of AED consumption in people with ischemic stroke using the Victorian linked health database.
Methods. Patients aged ≥30 years old and discharged from Victorian hospitals following ischemic stroke between July 2012 and June 2018 were included. The average Daily Defined Dose (DDD) of AED dispensed per person per day during the 1-year follow-up after discharge was estimated. Generalised linear model was used to examine the predictors of the AED consumption.
Results. Overall, the average DDD per day per person did not change significantly from 2012/13 to 2016/17 (Annual Percentage Change [APC], -2.4; p=0.3520). The utilisation of narrow spectrum (APC, -9.2; p=0.0164) and first-generation AEDs (APC, -7.7; p=0.0152) significantly declined over time. Compared to people aged 30-59 years, those aged >85 years were associated with higher consumption of AEDs (coefficient [β], 0.23; p=0.0037). Having neurological comorbidies such as epilepsy (β, 0.30; p=0.0001), bipolar disorder (β, 0.47; p=0.0032), schizophrenia (β, 0.57; p=0.0005), dementia and alzheimer's disease (β, 0.31; p<0.0001) were positively associated with AED consumptions. AED types such as lamotrigine (β, 0.28; p=0.0149), levetiracetam (β, 0.49; p<0.0001), valproate (β, 0.19; p=0.0430), phenytoin (β, 1.13; p<0.0001) or polytherapy (β, 0.56; p<0.0001) were positively associated with AED consumptions. Also, people with prior dispensing of AEDs were associated with higher AED consumptions than those receiving AED for the first time (β, 0.60; p<0.0001).
Discussion. There has been a trend towards receiving newer generation and broad-spectrum AEDs over older generation and narrow-spectrum AEDs. Older age, female gender, neurological comorbidities, history of AED and certain types of AEDs were associated with higher AED consumptions within 1 year following ischemic stroke. Future research is needed to assess the effectiveness of AEDs in controlling post-stroke seizures and epilepsy.

520 Prevalence and association of antipsychotic drugs with falls in hospitalised older adults

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Introduction. National and international guidelines have been developed to reduce inappropriate prescribing of antipsychotics.
Aims. To examine use of antipsychotic drugs in hospitalised older adults and investigate association with falls.
Methods. This was a retrospective audit of the medical records and casemix data of adults aged ≥65 years admitted to the two acute geriatric wards and one neurology ward between July and December 2020 at a tertiary hospital in Australia.
Results. Of the 998 study patients 46.2% were aged ≥85 years, 54.4% female and 39.5% had pre-existing cognitive impairment or delirium. While 154 patients (15.4%) were charted at least one antipsychotic drug, 104 (10.4%) patients had the antipsychotic drug administered during their hospitalisation. Among the administered antipsychotics, the most common were risperidone (31.9%), olanzapine (26.7%) and haloperidol (22.4%). Sixty-two (6%) patients had at least one fall in hospital. Administration of an antipsychotic drug was significantly associated with falls in hospital (OR = 2.74, 95% CI: 1.45-35.17, p=0.003). In multivariable logistic regression models adjusting for age, sex and pre-existing cognitive impairment/dementia, patients administered an antipsychotic drug (adjusted OR = 2.05, 95% CI: 1.05-4.00, p=0.036), and patients with pre-existing cognitive impairment/dementia (adjusted OR = 2.04, 95% CI:1.16-3.59, p=0.013) were twice as likely to have a fall.
Discussion. Antipsychotic drug use was low, suggesting that guidelines for minimising antipsychotic use in older people are influencing prescribing. Even so we still observed an association between administration of an antipsychotic drug and falls. There is an important role for ongoing medication review and interventions to reduce inappropriate prescribing of antipsychotics in hospital.

521 Trends in incidences and mortality of hip fractures and associated risk factors

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Introduction. Hip fractures are a major public health concern in Australia due to the ageing population. Contemporary data on hip fracture trends and risk factors is needed to inform healthcare resource allocation.
Aims. To examine the trend of hip fracture incidences and subsequent mortality and the risk factors associated with mortality among adults in Victoria, Australia between 2013-2018.
Methods. A population-based cohort study included all patients aged 30 years or older discharged from hospital following hip fracture between July 2012 and June 2018 in Victoria, Australia. Patients with a history of hip fractures within 6 years prior to their admission were excluded. Data including age, sex, frailty status, geographical location and residence (residential aged care facility [RACF] or not) were collected. Multivariate negative binomial regression was used to examine the trend and the effect of associated risk factors on incidence, 30-day and 1-year mortality.
Results. 32,395 patients with hip fractures were identified. No significant changes in incidence and mortality were found across the studied period after adjusting for age and sex. Several factors were found to be associated with increased 30-day mortality, including older age (≥85 years old versus 30-64 years old, risk ratio [RR]: 8.72, 95% confidence interval [CI] 6.48-11.96), male sex (RR 2.21 95% CI 1.97-2.47), higher frailty scores (high frailty versus low frailty, RR 1.68, 95% CI 1.37-2.07), residing in non-metropolitan region (RR 1.18, 95% CI 1.05-1.33), and residing in a RACF before admission (RR 2.49, 95% CI 2.03-3.05). The same factors were associated with increased risks of 1-year mortality; older age (≥85 years old versus 30-64 years old RR 25.01, 95% CI 19.46-32.13), male sex (RR: 2.47, 95% CI 2.16-2.81), higher frailty scores (high frailty versus low frailty RR 5.62, 95% CI 4.5-7.03), residing in non-metropolitan region (RR 1.39, 95% CI 1.22-1.59), and residing in residential aged care facilities before admission (RR 3.20, 95% CI 2.47-4.18).
Discussion. Standardised hip fracture trends have remained stable across the study period, yet absolute numbers have increased. Several factors were associated with increased 30-day and 1-year mortality. Future research is needed to determine if acting on the factors associated with increased mortality can reduce the overall burden on the healthcare system and society.

522 Medicine use in people with cystic fibrosis before and after modulator therapy

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Introduction. People with cystic fibrosis (PwCF) experience high treatment burden due to multiple pathologies and complexity of medicine regimens. Novel modulator therapies have proven efficacy in improving lung function and reducing pulmonary exacerbations, however impact on other subsidised medicine use is yet to be determined.
Aims. To investigate the total number and type of medicines used before and after the initiation of a novel modulator in PwCF.
Methods. A 10% random sample of people accessing medicines from the Australian Pharmaceutical Benefits Scheme (PBS) between July 2012 - May 2022, was used to identify PwCF who had received a modulator and had a prescription history for at least 90 days before and after modulator initiation. General linear models were use to compare total and type of medicines dispensed pre- and post-modulator.
Results. A total of 185 PwCF (Female 45%, median age 23 years (IQR 14-34)) who used a modulator were identified from 459 PwCF in the total random sample. A median of 3 (IQR 2-5) different medicines accessed in each 90-day period was observed. A reduction in total medicine count was detected however this did not reach statistical significance. Medicine type did not significantly change.
Discussion. This first real-world study of national dispensing data in Australian PwCF, demonstrated a non-significant reduction in total subsidised medicine use after modulator initiation. PwCF require multiple nonsubsidised treatments and future studies are required to evaluate total treatment use, as well as long term effects of modulators on medicine use and outcomes in PwCF. This study will aid modulator impact evaluation as further modulators become available and access for PwCF is expanded.

523 Can we accurately predict future dementia in middle-aged adults?

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Introduction. The ability to accurately predict future dementia may help select high-risk adults in middle-age and allow for targeted risk reduction to reduce the future incidence of dementia.
Aim. To appraise predictive accuracy of multi-domain prognostic models used in middle-aged adults to predict future dementia.
Methods. We searched 5 databases for developmental and validation studies of prognostic models; involving ≥2 modifiable risk factors of dementia (less education, hearing loss, traumatic brain injury, hypertension, alcohol intake, obesity, smoking, depression, social isolation, physical activity, diabetes mellitus, air pollution, diet & cognitive activity), used in middle-aged adults (45 - 65 years), for the prediction of subsequent dementia. We meta-analysed C-statistics when ≥2 studies for a model were identified, with a score >0.75 considered as good accuracy to differentiate people with and without dementia. We assessed risk of bias and applicability for all studies, and rated our overall confidence in findings.
Results. We identified 20 eligible studies with 14 unique prediction models. Four models had been externally validated, 10 had not. Hypertension and obesity were the most common modifiable risk factors. Two models were meta-analysed, Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) and LIfestyle for BRAin Health (LIBRA). The summary C-statistics of CAIDE was 0.73 (95% CI 0.68 to 0.77, 4 studies) and LIBRA was 0.67(95% CI 0.55 to 0.78, 3 studies). Overall the quality of the evidence was considered low due to high risk of bias and heterogeneity.
Discussion. While CAIDE demonstrated better accuracy than LIBRA, the low-quality evidence and poor accuracy means the benefits of using them in clinical practice to predict dementia later in life are unclear. The models may be of value for identifying potential participants for clinical trials of dementia risk reduction interventions. This Cochrane review highlights the need for further external validation of models using robust methodology and reporting guidelines.

524 Opioid deprescribing interventions for patients at transitions of care: a scoping review

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Introduction. The continuation of opioid therapy is not always warranted at transitions of care and it may increase the risk of adverse events. Studies have shown that the complexity of interventions is associated with increased effectiveness for improving appropriate opioid use. To our knowledge, no review has been conducted examining the impact and complexity of opioid deprescribing interventions at transitions of care.
Aims. To assess the impact, complexity, effectiveness and implementability of opioid deprescribing interventions at transitions of care.
Methods. We conducted a scoping review with a systematic search strategy according to 2020 PRISMA guidelines based on three concepts: opioid AND transitions of care AND deprescribing. The search was conducted in MEDLINE, EMBASE, Cochrane Library, CINAHL, PsycINFO, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, World Health Organisation International Clinical Trials Registry Platform and International Pharmaceutical Abstracts from inception to June 2022. Studies in English that focused on adults transitioning from one care setting to another, undergoing any deprescribing intervention with an intention to reduce or cease opioids, targeting patients, clinicians, or health system level were included. The studies will undergo analysis and evaluation by applying the 'Cochrane Intervention Complexity Assessment Tool for Systematic Reviews' (iCAT_SR), the 'Reach, Effectiveness, Adoption, Implementation, Maintenance' (RE-AIM) framework, and risk of bias will be assessed using either ROB-2 or ROBINS-I.
Results. On preliminary analysis, of 12130 reports screened, 211 studies were included with 70 (33%) being randomised controlled studies and 141 (67%) being observational studies. 137 studies (64%) of the results examined the transition from surgery to the ward, and 48 (22%) from hospital to home. Of the studies included, non-opioid analgesia (e.g. pregabalin, and gabapentin) was the most common intervention of opioid deprescribing, followed by multimodal pain management (e.g. multidisciplinary interventions, enhanced recovery after surgery protocols).
Discussion. Our preliminary results provide an insight into the diversity of pharmacological and non-pharmacological deprescribing interventions in a variety of transitions of care settings.

525 Audit of Data Sharing by Pharmaceutical Companies for Anticancer Medicines

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Introduction. Individual participant data (IPD) sharing enriches the post-approval evidence-base, informs future study designs, and facilitates novel secondary analyses. Since 2014, the pharmaceutical industry has acknowledged the importance of IPD sharing via endorsing their commitments to transparent IPD sharing with qualified researchers (i.e., >95% of big-pharma companies have an IPD sharing policy).
Aims. This study examined whether the IPD for key oncology trials supporting the registration of anticancer medicines approved within the past decade were eligible for sharing.
Methods. Pivotal trial results supporting FDA-approved anti-cancer medicines within the past decade were identified from product labels. The main outcome was frequency of IPD sharing eligibility. IPD sharing eligibility was confirmed by identification of a public listing of the trial as eligible for sharing or a positive response to a standardised enquiry to the sponsor.
Results. From 2011 to 2021, 115 industry-sponsored anticancer medicines were approved by the FDA, based on the evidence of 304 industry-sponsored trials. Of the 304 trials, 136 (45%) were eligible for IPD sharing. Data sharing rates differed substantially between industry sponsors. The most common reason (89 of 168 trials) a trial would not be shared was that the collection of long-term follow-up data was continuing. Of the top 10 anticancer medicines by global sales, nivolumab, pembrolizumab, and pomalidomide had the lowest eligibility rates for data sharing (< 10% of trials).
Discussion. There has been a significant increase in IPD sharing for industry-sponsored oncology trials over the past 5 years. However, it was found that >50% of queried trials for FDA-approved anticancer medicines were ineligible for IPD sharing. Data accessibility would be substantially improved if, upon registration of a medicine, all data supporting the registration was made available.

526 Between patient variability in drug disposition of large vs small molecules

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Introduction. The dose-response relationship varies between- and within-individuals. One area of between-subject variability (BSV) is in drug disposition and is usually caused by various patient variables such as age, body size, and organ function. Such variability necessitates a multi-fold difference in dose requirements, particularly for drugs with a narrow therapeutic window. Accurate identification of the sources of variability and quantification of their magnitude is expected to improve the precision of drug dosing. Previous work has shown that, across a range of drugs and population pharmacokinetic studies, the mean variability (expressed as coefficient of variation, CV%) of clearance (CL) of small molecule drugs was 40.3% (IQR 26-48).[1]
Aims. To explore the range of BSV values in PK parameters of large molecule drugs in patient populations.
Methods. A literature review of population PK studies was conducted. Estimates of clearance (CL) and other model parameters and their corresponding BSV (CV%) were recorded.
Results. A total of 112 adult popPK studies involving 78 drugs and 23 paediatric popPK studies involving 22 drugs were identified. In adult studies, the median CV% in CL/F was 31% [IQR 24-38]. The median CV% in CL/F in paediatric studies was 28% (IQR 21-34). Disease state, age, and body size were among the most significant covariates reported.
Discussion. BSV in drug clearance of large molecules appears to be lower than that of small molecules. This may be related to the elimination pathways of these drugs and/or the existence of very influential covariates that account for the variability. Furthermore, the influence of this variability on dose requirements is limited by the relative safety of large molecules in high doses and the variability in the physiological response.

[1] Al-Sallami et al. EJCP 2014; 70(11):1403-4

527 Suppressing antibiotic resistance of hypermutable *Pseudomonas aeruginosa* clinical isolates with an inhaled aztreonam and tobramycin combination dosing regimen in a dynamic *in vitro* biofilm model

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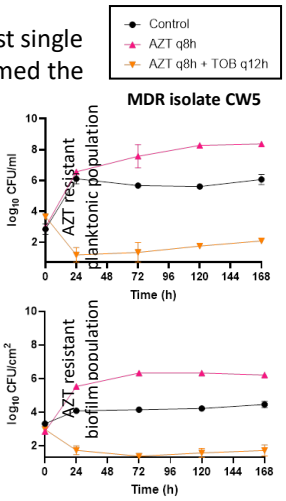
Introduction. Antibiotic resistance is one of the greatest threats to humans, an issue which is exacerbated by suboptimal antibiotic regimens increasing occurrence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* (Pa), and the prevalent hypermutable Pa strains in patients with cystic fibrosis (CF). Ensuring effective antibiotic use is essential.
Aims. To evaluate resistance emergence following aztreonam (AZT) and tobramycin (TOB) inhaled dosing regimens against clinical hypermutable Pa strains in a dynamic *in vitro* biofilm model (BF).
Methods. Two CF MDR strains were studied in a 168h BF (n=2; inoculum $10^{5.5}$ CFU/mL) against single and combined inhaled AZT (75mg, q8h) and TOB (300mg, q12h) regimens. LC-MS/MS confirmed the simulated PK of AZT and TOB, based on published PK of lung fluid concentrations in CF patients ($t_{1/2}$=3h). Total viable counts and resistant bacteria were determined for planktonic and biofilm bacteria. Bacterial counts were mathematically modelled (MBM). MICs of resistant populations were determined after the BF.



Results. Reproducible results for both isolates showed amplification of resistance and bacterial regrowth for the monotherapies by 168h. Resistance was sustained in further testing. The combination was synergistic (>2 $\log_{10}$ CFU/mL or CFU/cm^2 more bacterial killing compared to the best monotherapy and the initial inoculum) against planktonic and biofilm bacteria of both isolates at 168h, with minimal resistant subpopulations. Subpopulation and mechanistic synergy well described the antibacterial effects of the combination regimen in the MBM.
Discussion. Across the biological replicates of both of the hypermutable MDR Pa isolates from patients with CF, the combination of AZT and TOB was required to suppress regrowth and resistance of the planktonic and biofilm bacteria. Further investigation of this promising synergistic combination dosing regimen is warranted.

528 Investigating the phenotypic plasticity of glioblastoma stem cells

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Introduction. Glioblastoma is a fatal brain tumour. Standard-of-care chemotherapy with temozolomide (TMZ) provides minimal therapeutic relief, as cell cycle arrest reverses after treatment and the cells reproliferate. This is called phenotypic plasticity, a hallmark of glioblastoma stem cells (GSCs), and allows glioblastoma cells to survive treatment and recur. Novel combination therapies which would disable plasticity offer a promising option for improving glioblastoma standard-of-care.

Aims. To assess whether TMZ induces reversible cell cycle arrest in GSCs and characterise the associated phenotype, by identifying the mechanisms which regulate GSC phenotypic plasticity.

Methods. To characterise the GSC phenotype, GSCs were with TMZ and compared them to parentals through RNA sequencing, qPCR, western blotting, immunofluorescence and β-galactosidase staining. To examine the reversibility of TMZ-induced cell cycle arrest, TMZ-treated cells were placed in drug-free holiday and analysed using clonogenics and IncuCyte live cell imaging.

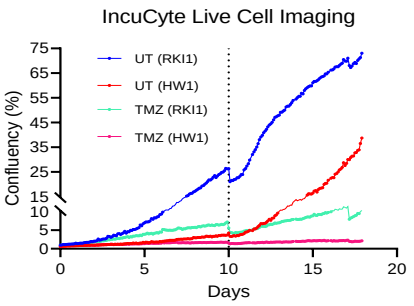

Figure 1. IncuCyte quantification of cell confluency (%) during and after (separated by line) treatment with temozolomide (TMZ, 25 µM, n = 1).

Results. TMZ induces reversible cell cycle arrest in GSCs as seen by reduction in clonogenicity and proliferation, before cells recover in drug holiday. Hallmarks of senescence and quiescence are present in TMZ-treated GSCs, contributing to GSC phenotype. Epigenetic modifiers are upregulated in TMZ-treated GSCs (KMT3D, KDM5B and KDM7A). RNA sequencing identified genes regulating lipid metabolsim and stemness may contribute to phenotypic plasticity.

Discussion. Reversible cell cycle arrest in TMZ-treated GSCs implicates phenotypic plasticity in response to chemotherapy. The presence of both senescence and quiescence markers suggests that GSC cell populations modulate their phenotype heterogeneously in response to TMZ. Epigenetic reprogramming may potentially contribute to the transient nature of GSC cell cycle arrest.

529 Using recombinant DNA technology to produce a delivery system for genetic drugs

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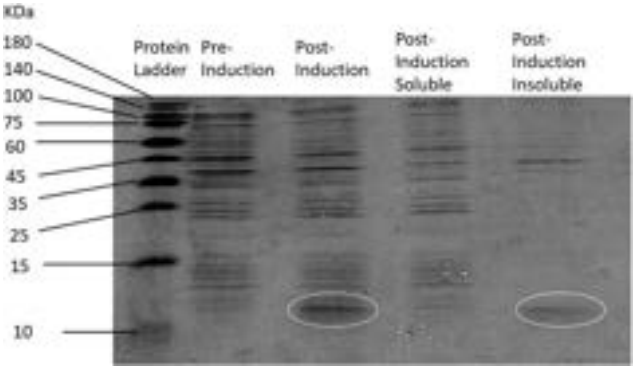
Introduction. Gene therapy is widely studied to treat multiple diseases. One of the barriers to this type of therapy is the ability to deliver genetic material into target cells/tissues. To increase the cell/tissue specific uptake of gene therapy, the development of targeted delivery systems is necessary. In this study, we aimed to use a tumour-targeting cell penetrating peptide, along with an endosome escape peptide and a genetic material binding peptide, to form a targeted gene delivery system for production using recombinant DNA technology.



Aims. A commonly used peptide production method in labs is solid-phase peptide synthesis (SPPS). However, this technique suffers from high cost and low yield when producing long peptides. Thus, in this study, we aimed to use recombinant DNA technology, which is widely used in the biotechnology production facilities, to improve the capacity to scale up production of our gene delivery systems.

Method. In this study, a gene sequence coding for our delivery system was codon optimised, purchased from Integrated DNA Technologies (IDT), and restriction cloned into a plasmid (pET-28b) for expression in BL21(DE3) *Escherichia coli (E. coli)*.

Result. Four gene sequences, corresponding to different orders of each peptide component, were successfully cloned into pET-28b and demonstrated to be capable of expression in BL21(DE3) *E. coli*.

Discussion. The capacity to express each of these delivery systems in *E. coli* demonstrates our potential to transition production of these delivery systems from synthetic to recombinant processes, offering improved ability to scale up production for clinical translation, and reduced production costs.

530 Metabolic footprinting in a hollow-fibre infection model: ceftolozane-tazobactam *versus Pseudomonas aeruginosa*

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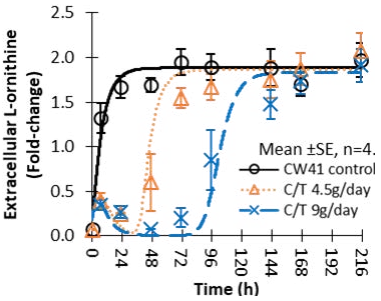
Introduction. Using extracellular metabolites in an *in vitro* PK/PD study like a hollow-fibre infection model (HFIM) could expedite the quantification of bacterial response to antibiotic exposure compared to traditional microbiological culturing protocols. Ceftolozane-tazobactam (C/T) is not yet approved for patients with cystic fibrosis (CF) and relies on off-label dosing regimens.
Aims. To mathematically model the relationship between bacterial response and extracellular metabolites in a HFIM.
Methods. A C/T-susceptible and multidrug-resistant hypermutable *Pseudomonas aeruginosa* CF clinical isolate, CW41, was challenged with C/T concentrations simulating continuous infusions of standard (4.5g/day) and high (9g/day) daily doses in the HFIM for 7-9 days (n=4). Ceftolozane concentrations were confirmed by LC-MS/MS. Total and resistant bacterial populations were quantified and mathematically modelled. Spent supernatant from HFIM was analyzed with untargeted LCMS-based metabolomics, and correlation analysis with bacterial data. Selected metabolites were co-modelled with their respective correlating bacterial population with a PK/PD-based transduction model.
Results. Both doses of C/T provided some killing, then failed with amplified resistance from 48-72h onwards. Secreted L-ornithine (Figure) and assimilated L-arginine highly correlated with the total bacterial population (0.82 and -0.79 respectively, p<0.0001). Ribose-5-phosphate, sedoheptulose-7-phosphate and trehalose-6-phosphate correlated with the resistant subpopulation (0.64, 0.64 and 0.67, respectively, p<0.0001), and were likely secreted as a result of resistant growth overcoming oxidative and osmotic stress induced by C/T exposure.
Discussion. Five extracellular metabolites were well described with mathematical modelling based on bacterial response. This proof-of-concept study suggests further exploration with other antibiotics and *P. aeruginosa* strains is warranted. Ceftolozane-tazobactam should be administered in combination with other antibiotics for CF patients, aligning with current CF treatment guidelines.



531 PK/PD indices do not predict the outcome of ciprofloxacin *versus Pseudomonas aeruginosa* with different resistance mechanisms

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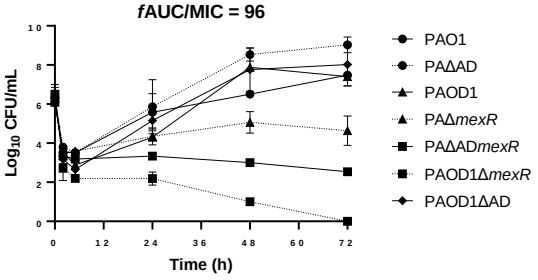
Introduction. PK/PD indices are based on minimum inhibitory concentrations (MICs) and link response of bacteria to their exposure to antibiotics. The index most relevant for fluoroquinolone antibiotics is the ratio of free drug area under the concentration-time curve to MIC over 24 h (*f*AUC/MIC).
Aims. We tested if the effect of ciprofloxacin on isogenic bacterial strains of *Pseudomonas aeruginosa* with different resistance mechanisms is predicted solely by *f*AUC/MIC or depends on the mechanism of resistance.
Methods. Seven isogenic *P. aeruginosa* strains: PAO1 (wild-type reference strain), PAΔ*mexR* (*mexR* knockout/MexAB-OprM overexpression), PAOD1 (spontaneous *oprD* mutant/loss of porin OprD), PAΔAD (*ampD* knock-out/AmpC overexpression), and PAΔAD*mexR*, PAOD1Δ*mexR* and PAOD1ΔAD (strains with a combination of resistance mechanisms) were used. The ciprofloxacin MIC of each strain was determined. They were then exposed to constant concentrations of ciprofloxacin (0.5-4 mg/L) over 72 h.
Results. MICs were 0.125-1 mg/L. *f*AUC/MIC of 48 suppressed regrowth of PAOD1Δ*mexR*. *f*AUC/MIC of 96 was needed against PAΔ*mexR* and PAΔAD*mexR* (Figure). *f*AUC/MIC of 192 supressed regrowth in PAΔAD, PAOD1, and PAOD1ΔAD. *f*AUC/MIC of 384 was needed to supress regrowth of PAO1.
Discussion. These studies indicated that *f*AUC/MIC alone did not predict the ciprofloxacin exposure required to suppress bacterial regrowth over 72 h. At the same *f*AUC/MIC, different resistance mechanisms might influence the ciprofloxacin exposure needed to suppress regrowth. The study indicates traditional PK/PD indices do not fully explain the relationship between antibiotic exposure and bacterial response, and mechanisms of resistance may need to be considered when optimizing antibiotic dosing.

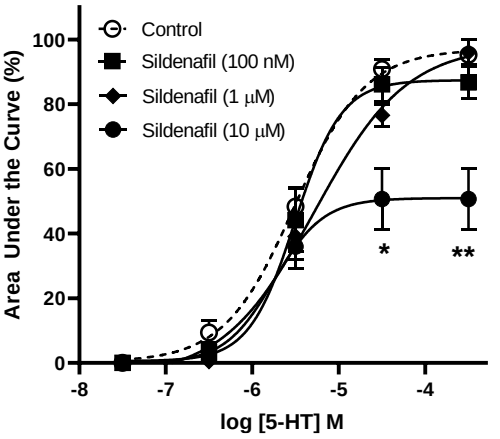


533 The effects of sildenafil on porcine distal ureteral contractions

Iris Lim[1], Russ Chess-Williams[1], Donna Sellers[1] Faculty of Health Sciences & Medicine, Bond University[1], Gold Coast, QLD, Australia.

Introduction. Recent clinical trials have suggested that the use of phosphodiesterase type 5 (PDE-5) inhibitors could increase stone expulsion rate in patients affected with urolithiasis (AbdelRazek et al 2022). This study aimed to investigate the effects of sildenafil on porcine ureteral contraction, which has been shown to be a close pharmacological and physiological model to humans (Lim et al .

Methods. Contractile responses of isolated porcine distal ureteral strips to 5-HT were examined in the absence and presence of sildenafil (100 nM, 1 μM, 10 μM). In another set of experiments, tissue strips were pre-contracted with 5-HT (300 μM) followed by a concentration response to sildenafil (1 nM – 10 μM). When subjected to increasing concentrations of 5-HT, porcine ureteral tissues developed bursts of phasic contractions, and these responses were expressed as area under the curve, taking into account frequency and amplitude of the contractions.



Results. Sildenafil, at lower concentrations of 100 nM and 1 μM did not affect 5-HT-induced contractile responses in the ureter. In the presence of sildenafil (10 μM), the 5-HT induced responses were reduced ($p < 0.05$). In the concentration response curves to sildenafil, only concentrations of 1 μM and higher induced a significant relaxation.

Discussion. The present findings suggest that sildenafil has an attenuation effect on porcine ureteral contraction at high concentrations, but not at lower concentrations where it is selective for PDE-5. It is unlikely that sildenafil induces this effect via inhibition of PDE-5 enzyme, but via a different phosphodiesterase enzyme or a different mechanism.

AbdelRazek M et al (2022) Worl J Urol 40(8):2063-2070
Lim et al (2020) J Pharmacol Toxicol Methods 102:106661

534 Severe hyperglycaemia impairs bladder contractility in a murine model of diabetes mellitus

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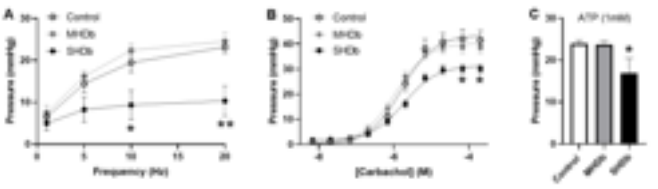
Introduction. Diabetic bladder dysfunction is extremely common in patients and thought to progress from overactive bladder to decompensation (Panigrahy et al, 2017), although mechanisms underlying the progression are not clear.

Aims. To examine the effects of hyperglycaemia on bladder physiology in a murine model of type 1 diabetes mellitus.

Methods. Female C57BL/6J mice (12-14 weeks) received streptozotocin (*i.p.* 50mg/kg) or citrate buffer daily for 5 days. At day 16 mice were euthanised, blood glucose measured and whole bladders isolated in Krebs-bicarbonate solution (37°C, 95%O_2/5%CO_2) to measure spontaneous activity, bladder accommodation, and contractile responses to electrical field stimulation (EFS) and pharmacological agents.

Results. Animals were stratified according to blood glucose levels, control <10mM (8.9±1.4mM, n=6), mild hyperglycaemia 10-15mM (MHDb, 12.9±2.0mM, n=7) and severe hyperglycaemia >20mM (SHDb, 24.6±5.9mM, n=4). Severe hyperglycaemia impaired contractile responses to EFS at 10 and 20Hz relative to controls and MHDb (Fig. 1A, $P<0.05$, $P<0.01$, one way ANOVA plus Tukey's). Relative contributions of ATP, ACh and NO to nerve-mediated responses were unaltered. Maximal responses to carbachol and ATP were also significantly reduced in the SHDb group vs controls and MHDb (Fig. 1B,1C, $P<0.05$), whilst contractions to high KCl, spontaneous activity and bladder accommodation following filling were unaltered and similar between groups.

Discussion. Severe hyperglycaemia impairs key control mechanisms involved in bladder contraction (neuronal, cholinergic and purinergic responses) without a change in overall bladder contractility, changes that are not observed with mild hyperglycaemia. These changes may partly explain the decompensation and decreased sense of bladder fullness experienced by patients.

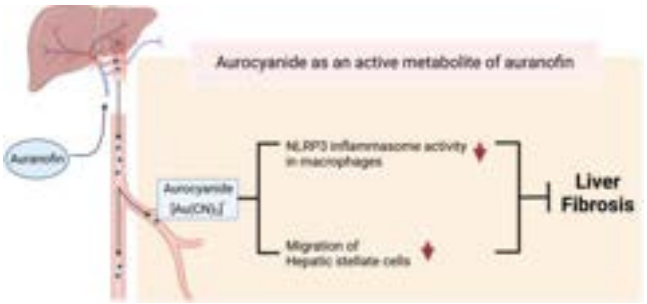


Panigrahy R et al 2017 Diabetes Metab Syndr 11:81–82

535 Gut dysbiosis in refractory hyperammonaemic syndrome post lung transplant: bystander or bad guy?

Tess Evans[1,4,5], Ed Raby[3,4], Oliver Waters[2,4], Ryan Anderton[5], Laurens Manning[3,4], Ed Litton[1,4]. Departments of Intensive Care[1], Gastroenterology[2] and Infectious Diseases[3], Perth, Western Australia, Australia; School of Medicine[4], University of Western Australia, Perth, Western Australia, Australia; Faculty of Health Sciences[5], University of Notre Dame, Fremantle, Western Australia, Australia

Introduction. Overabundance of opportunistic pathogens is a hallmark of dysbiosis in critical illness. Transplant patients are highly susceptible due to severe immune suppression with exposure to multiple broad-spectrum antimicrobials. Although the pathophysiological determinants are unclear, urea-splitting organisms colonise 26% of lung transplant patients, where 1-4% of recipients will develop hyperammonaemic syndrome, a high mortality (76%) condition frequently co-occurrent with dysbiosis.



Aims. We present a hypothesis-generating case of refractory hyperammonaemic syndrome after clearance of donor derived infection in the setting of severe *Pseudomonas* predominant dysbiosis. Presentation and Management. A 70-year old male, SV, developed hyperammonaemic encephalopathy in the context of a donor-derived *Ureaplasma urealyticum* and *Mycoplasma spp.* infection post bilateral sequential lung transplant with prompt recognition and treatment. Despite initial improvement SV developed recurrent gastrointestinal bleeding of largely non-specific focus, non-surgical abdominal pain, sepsis and diarrhoea with *Pseudomonous aeruginosa* and non-difficile *Clostridium* bacteraemias cultured over the subsequent 6 weeks. No unifying diagnosis was found after radiographic, serial panendoscopic and histopathological evaluation and complications of critical illness accrued. He received over 15 antimicrobial agents including 6 weeks of doxycycline/azithromycin.

Outcome. In his 7th week, SV suffered a relapse of hyperammonaemic syndrome with grade III encephalopathy with no recurrence of the initial pathogens detected; invasive treatment was withdrawn in the setting of ongoing deterioration on day 80. 16S rDNA PCR of stool sampled at week 8 later confirmed clinical suspicion of ultra-low diversity, with marked dominance of *Pseudomonas* spp. which was later cultured from sputum, skin and blood.

Discussion. The relationship between hyperammonaemia and dysbiosis in the transplant population is unknown. Antimicrobials appear to serve as an ecological selection pressure fostering an overgrowth of opportunistic pathogens sustained by physiologic stress and other modulators. The finding of intercurrent *Pseudomonas* dominance and hyperammonaemia raises the possibility of urea-splitting in vivo. Supporting lines of evidence and include presence of urease in approximately 50% of *Pseudomonas* isolates and positive urease breath tests in *Pseudomonas* colonised CF patients. Large prospective observational studies integrating next-generation sequencing of gut and lung microbiome samples would inform the interaction between dysbiosis and complex infectious and metabolic complications in vulnerable ICU populations. Trials of faecal microbiota transplant with treatment-resistant, dysbiosis-related conditions warrant exploration.

536 Aurocyanide is an active metabolite of auranofin for its anti-liver fibrosis effect

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Introduction. During the past several years, the interest in drug repositioning has greatly increased and auranofin, an anti-rheumatoid arthritis drug, is also being investigated for potential therapeutic applications in other diseases including liver fibrosis. However, active metabolites of auranofin remain elusive, making it difficult to estimate its clinical efficacy.

Aims. We tried to identify active metabolite(s) of auranofin for its anti-fibrotic effect.

Methods. Bone marrow-derived macrophages were used to evaluate the inhibitory effects of assigned metabolite compounds on system x_c^- and NLRP3 inflammasome. Pharmacokinetics parameters and *in vivo* anti-fibrotic effects of aurocyanide were assessed in mice. The *in vitro* anti-fibrotic effects of aurocyanide were assessed in LX-2 cells.

Results. Incubation of auranofin with liver microsomes indicated that auranofin is susceptible to hepatic metabolism. System x_c^--mediated inhibition of NLRP3 inflammasome contributes to the anti-fibrotic effect of auranofin. Among the 7 metabolic candidates, 1-thio-β-D-glycopyrano-sato-S-(triethyl-phosphine)-gold(I) and aurocyanide potently suppressed both system x_c^- and NLRP3 inflammasome activities. Mouse pharmacokinetic study revealed that reliable amounts of aurocyanide were detected in plasma after administration with auranofin. Oral administration of aurocyanide significantly prevented thioacetamide-induced liver fibrosis in mice. Moreover, aurocyanide markedly decreased the migratory ability of LX-2 cells.

Discussion. Based on the molecular mechanism that underlies its anti-fibrotic effect, we suggest aurocyanide as an active metabolite of auranofin.

537 Implementation of global gut sampling in the Prospective Observational Embedded Microbiome Study (POEMS)

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Introduction. A prospective single-centre observational cohort study will be conducted at the 40-bed Intensive Care Unit at a quaternary hospital in Perth, Western Australia over a 24month recruitment period. All patients admitted to the ICU over the study period will be included by waiver of consent; estimated *n*=5000. Leveraging usual care, rectal swabs collected for Vancomycin Resistant Enterococcus (VRE) screening will undergo batched metagenomic sequencing of 16S rDNA amplicons. Clinical, biochemical and physiologic data will be extracted from the medical records to ascertain all exposure and outcome variables.
Aims. To review protocol adherence, facilitators and barriers from implementation of a novel, embedded study design.
Method. The ICU Clinical Information System allows automated extraction and reporting of all of participants including missed admissions. For qualitative assessment, verbal, and written feedback was sought from all stakeholders as well as time-in-motion observation of sampling.
Results and Discussion. Eight weeks post study commencement more than 80% of admission samples and 60% of discharge samples were captured for POEMS per fortnight, enabling meaningful longitudinal comparisons. Facilitators included early engagement of clinical nurse educators, nurse unit managers, forming a mutually agreed staggered timeline for multi-modal dissemination of study protocol elements to research and registered nurses, medical officers, administrative staff and enrolled and assistant nurses. Use of equipment displays, graphics and photographs as well as strong physical presence in the unit were reported as enablers of the protocol. Significant investment in education and the ethos of embedded research from nursing leadership has maintained adherence despite high staff and patient turnover. Barriers included stock shortages, storage arrangements including time-dependent transfer to -80degree freezer. Study procedures have been adapted and will be continually reviewed to ensure ambitious recruitment targets are met, with particular attention to ICU discharge. It is anticipated POEMS serves as a favourable, readily scalable precedent for other sites as well as further embedded approaches within the study site.

538 Serious statin associated myotoxicity (SSAM) in Indigenous Australians – a literature case series 2010-2020

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Introduction. Statins are widely used for cardiovascular risk reduction and rarely associated with serious myotoxicity.
Aims. To review reported cases of SSAM in Indigenous Australians; to inform future risk mitigation strategies in a high cardiovascular risk population.
Methods. Cases identified from literature (Embase, PubMed, CINAHL, TROVE, Google Scholar) from Jan 2010-Dec 2020, and spontaneous reports from Database of Adverse Events Notifications (DAEN), TGA. Demographic, clinical features, co-morbidities, statin exposure, outcome and treatment were extracted.
Results. 42 cases were identified, 40 from literature, 2 from DAEN. Cases were from SA/NT (n=26), Qld (n=12), NSW (n=2) and WA (n=1). There were 26 females and 16 males, mean age 55 years (20-69). Cases were identified as Aboriginal, Aboriginal and Torres Strait Islander or Indigenous. No information on language group was reported. Co-morbidities (21 cases) were diabetes (n=16), hypertension (n=14) and low Vitamin D (n=14). Established cardiovascular disease (CVD) was infrequent (n=3). Complete drug information including statin type, dose, duration of exposure and other medications was available for only 7 cases. Statins were atorvastatin (n=28), rosuvastatin (n=2), simvastatin (n=1), unknown (n=11). Duration of prior statin exposure was 3 weeks to 13 years. Presentation was with weakness (n=35), myalgia (n=11) or dysphagia (n=7). Onset of symptoms was over 1 to 3 months. Mean peak creatine kinase was 51,335 (R 23-580 000 U/L). Anti-HMGCoA reductase antibodies were positive in 7 of 16 tested. Biopsy (n=31) showed immune mediated necrotising myositis (27), polymyositis (3) or nonspecific inflammatory myositis (1). Outcomes, available for 25 cases, were complete resolution (n=11), ongoing disability (weakness) (n=8) or death (n=7).
Discussion. 42 cases of SSAM in Indigenous Australians were identified, mostly in individuals without established CVD. Information in case reports was incomplete regarding drug exposures and case language groups. Geographic distribution of cases does not reflect population distribution potentially suggesting insufficient understanding and/or under-reporting of adverse drug outcomes. Improved clinical identification, investigation and reporting may better inform future risk mitigation strategies

539 Development of a PBPK model for Irbesartan in different *CYP2C9* genotypes

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Introduction. Irbesartan selectively and noncompetitively binds to the angiotensin II (AII) receptor subtype 1 (AT1) inhibiting the activity of AII. Irbesartan effectively lowers BP in patients with hypertension (see Therapeutic Use summary) without affecting heart rate. It is mainly metabolized to inactive metabolites by CYP2C9. CYP2C9 is genetically polymorphic, and it has been reported that *CYP2C9*3* and **13*, the main variant alleles in East Asians, are related to the decreased metabolism of irbesartan. .
Aims. For a pharmacotherapy strategy that minimizes individual differences in drug response according to individual physical and physiological characteristics and genotype of *CYP2C9*, a PBPK model of irbesartan was developed and validated in different *CYP2C9* genotypes.
Methods. PBPK model of irbesartan was developed using the PBPK modeling software PK-Sim® version 10.0. Parametric optimization was performed using the Levenberg – Marquardt algorithm implemented in PK-Sim®. The pharmacokinetic data (12 CYP2C9**1/*1*, 10 CYP2C9**1/*3*, and 6 CYP2C9**1/*13*) of our previous pharmacogenomic study was used to develop the PBPK model.
Results. The input value of each parameter for PBPK model development is shown in Table. With these input values, the PBPK model of irbesartan was well constructed in different CYP2C9 genotypes. Validation was performed with PK data of 6 reports of other researchers, and the fold errors for AUC_{inf}, C_{max}, and $t_{1/2}$ were within acceptance criterion (two-fold).
Discussion. The PBPK model of irbesartan in different CYP2C9 genotypes was successfully established. This model can be used for individualized pharmacotherapy.

Parameters	Input value
Physicochemistry	
Molecular Weight (g/mol)	428.53
Fraction unbound (%)	10
Solubility (mg/mL)	129
*Reference pH 1.2	
Lipophilicity (log P)	2.7
Dissociation constant (pK_a)	5.80(acidic) 4.12(basic)
Metabolism	
CYP2C9 *wt/*wt	
Vmax (pmol/min/pmol)	46
K_m (μM)	54
CYP2C9 *1/*3	
Vmax (pmol/min/pmol)	27
K_m (μM)	57
CYP2C9 *1/*13	
Vmax (pmol/min/pmol)	24
K_m (μM)	57
UGT1A3	
Vmax (pmol/min/pmol)	30
K_m (μM)	368.6
Transport	
OATP1B1	
Vmax (pmol/min/pmol)	1
K_m (μM)	0.69
OATP1B3	
Vmax (pmol/min/pmol)	0.3
K_m (μM)	11.09
Formulation	
80% dissolution time (min)	10

540 PBPK modeling and simulation for glipizide related to *CYP2C9* genetic polymorphism

Pureum Kang[1], Hye-Jung Park[1], Chang-Keun Cho[1], Eunvin Ko[1], Chou Yen Mu[1], Choon-Gon Jang[1] and Seok-Yong Lee[1].
[1]School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea.

Introduction. Glipizide is a sulfonylurea antidiabetic drug used for the treatment of type 2 diabetes. It is mainly metabolized to the inactive metabolites by CYP2C9. CYP2C9 is genetically polymorphic, and its enzyme activity vary depending on the genetic variation. Therefore, the pharmacokinetics of glipizide are significantly different depending on the CYP2C9 genotype of the patient.
Aims. It is possible to develop a pharmacokinetic model that reflects differences in the genotypes of individual metabolic enzymes through the physiologically based pharmacokinetic (PBPK) model, which develops from the existing compartment model and predicts pharmacokinetics based on physiology. The goal of this study is to make it possible to predict pharmacokinetics taking into account individual characteristics, and to help with personalized pharmacotherapy.
Methods. The PBPK model was developed as a middle-out method combining top-down and bottom-up methods, and the physicochemical data of glipizide and ADME-related data were obtained from previous studies. The PK data of our previous pharmacogenomic study were used to develop the model.
Results. The input value of each parameter for PBPK model development is shown in Table. With these input values, the PBPK model of glypizide was well constructed in different CYP2C9 genotypes. Validation was performed with PK data of 7 reports of other researchers, and the fold errors for AUC_{inf}, C_{max}, and $t_{1/2}$ were within acceptance criterion (two-fold).

Parameter			Input value
Basic physical-chemistry			
Molecular weight			445.54 g/mol
Log P			2.5
Fraction unbound			0.08
pK_a			4.42 & 8.06
Solubility			0.02 mg/mL
Formulation			
Dissolution time (80%)			25 min
ADME (Absorption, Distribution, Metabolism, Excretion)			
Specific intestinal Permeability			4.92E-5 cm/s
Specific organ permeability			1.36 E-4 cm/min
OAT1B1	Reference conc.		1 pmol/L
	V_{max}		5 pmol/min/pmol transporter
	K_m		400 pmol/L
ABCB11	Reference conc.		1.71 pmol/L
	V_{max}		35 pmol/min/pmol transporter 11.2 pmol/min/pmol transporter
	K_m		88 pmol/L
CYP2C9	Reference conc.		2.5 pmol/L
HM CYP2C9 *1/*1	V_{max}		332.2 pmol/min/pmol rec. enzyme
	K_m		1.78 pmol/L
	K_{cat}		49.70 L/min
IM CYP2C9 *1/*3 & *1/*13	V_{max}		516.1 pmol/min/pmol rec. enzyme
	K_m		168 pmol/L
	K_{cat}		86 L/min
PM CYP2C9 *3/*3	V_{max}		1.78 pmol/min/pmol rec. enzyme
	K_m		79 pmol/L
	K_{cat}		1.78 L/min
Renal Clearance	Plasma Clearance		0.02 mL/min/kg
Biliary Clearance			0.026 mL/min/kg
Physiological parameters			
Gastric emptying time			10.45 min
S-I-C contained fraction			92%
Gallbladder ejection fraction			90%
Gallbladder ejection half-time			15 min
Event			
Gallbladder emptying event time			4.48 hr

Discussion. The PBPK model of glypizide in different CYP2C9 genotypes was successfully established. This model can be used for individualized pharmacotherapy.

541 Preventing urate-lowering therapy induced gout flares: a systematic review and network meta-analysis

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Introduction. Urate-lowering therapy (ULT) initiation may precipitate a transient rise in flares. However, evidence for the comparative rate of flares for different ULT therapies and drugs used to prevent flares (prophylaxis) is limited.
Aims. To examine: (1) flare risk post initiation or escalation of different ULTs; (2) change in flare risk with and without prophylaxis; (3) adverse event (AEs) rate associated with prophylaxis; and (4) the optimal duration of prophylaxis.
Methods. We searched Medline, Embase, Web of Science, Cochrane databases and clinical trial registries for clinical trials investigating adults with gout initiating or escalating ULT, from inception to Nov 2021. Frequentist random-effect network meta-analyses were performed and reported risk ratios (RR) with 95% confidence intervals (95% CIs). We assessed bias using the Revised Cochrane risk-of-bias tool. The study is registered with PROSPERO, CRD42020178479.
Results. We identified 3775 records, of which 29 publications (27 trials) were included. Comparative to placebo plus prophylaxis, the RR of flares ranged from 1.08 [95% CI 0.87-1.33] for febuxostat 40mg plus prophylaxis to 2.65 [95% CI 1.58-4.45] for febuxostat 80mg plus lesinurad 400mg plus prophylaxis. Due to a low level of reporting of flares per prophylactic drug in trials that did not randomise prophylaxis, comparisons of flare risk between prophylactic drugs were limited to trials where prophylaxis was randomised. ULT plus prophylaxis was associated with a lower RR of flares compared to ULT alone (0.35 [95% CI 0.25-0.50] rilonacept 160mg, 0.43 [95% CI 0.31-0.60] rilonacept 80mg and 0.50 [95% CI 0.35-0.72] colchicine). Rilonacept was associated with greater treatment related AEs relative to ULT alone. Most studies were assessed as a high risk of bias, with 4 (14%) of 28 studies and 2 (18%) of 11 studies rated as low risk of bias for flares and AEs, respectively.
Discussion. The risk of ULT-induced flares varied depending on the ULT drug and dosing strategy. Colchicine and rilonacept significantly reduced the incidence of flares, but the evidence for nonsteroidal anti-inflammatory drugs and corticosteroids was limited. Additionally, there was limited data on the harms and optimal duration of prophylaxis.

542 Development of Plasma Assay for Tyrosine Kinase Inhibitors

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Introduction. Tyrosine kinases inhibitors (TKIs) are orally administered antineoplastic drugs that target tyrosine kinases by blocking signalling pathways and inhibiting tumour cell growth. These drugs are commonly administered using a “one dose fits all” approach. The large interpatient variability in the pk of TKIs will result in patients who may be underdosed and others where the dose is too high. In renal cell carcinoma patients, pazopanib and sunitinib treatment has been established and is able to be measured and analysed using LC-MSMS. Future treatment is moving towards using other TKIs including lenvatinib, cabozantinib and axitinib. This begs the need for a validated method to accurately analysis these new TKIs in blood.
Aim. To develop an LC-MSMS analysis technique to measure six TKIs in plasma suitable for concentration monitoring of these TKIs; lenvatinib, imatinib, pazopanib, axitinib, sunitinib, and cabozantinib in the clinical setting.
Methods. 50µL of blank plasma samples, obtained from volunteers, was spiked with the six TKIs, with concentrations selected based on their therapeutic range. Plasma samples were extracted using acetonitrile containing an internal standard solution. Samples were vortexed and centrifuged, and the supernatant was injected into a Shimadzu 8060 LC-MSMS. Chromatographic separation was achieved on a Kinetex C18 column with a gradient elution of 0.1% formic acid in water and acetonitrile.
Results. The six TKIs studied had linear calibration curves ranging from lenvatinib (2 – 1000ng/mL), imatinib (100 – 2000ng/mL), axitinib (0.5 – 50ng/mL), pazopanib (1000 – 50000ng/mL), sunitinib (5 – 500ng/mL) and cabozantinib (50 – 15000ng/mL). Plasma samples r^2 values ranged from 0.98 – 0.99. The intra and inter-day imprecision ranged from 0.31 – 9.75 and 3.55 – 15.52% respectively.
Discussion. The development of validated analytical techniques to analyse TKIs provides the initial work to facilitate reliable measurement of TKIs for clinical use, making it readily available in laboratories across Australia.

543 Vanquish the vancomycin trough: the AUC strikes back

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Introduction. Therapeutic drug monitoring of vancomycin is recommended, with international guidelines advising a target AUC_{24h} of 400-600 mg/L.h for a minimum inhibitory concentration of ≤1 mg/L. Bayesian monitoring is also preferred for dose optimisation. Our tertiary institution recently switched from targeting trough concentrations to AUC_{24h}, using a Bayesian-based approach.
Aims. To describe and evaluate the AUC_{24h} obtained for initial and ongoing prescribed vancomycin doses, time to attain target AUC_{24h} and the relationship between trough concentrations and AUC_{24h}.
Method. Retrospective data were compiled from adult patients given vancomycin for at least 48 hours during eight consecutive months in 2021, following the change in local vancomycin guidance. These data included patient characteristics, prescribed doses and plasma vancomycin and creatinine concentrations, and are expressed as median (range).
Results. A total of 129 patients were included, with median age 62 (18-95) years and creatinine clearance 72 (13-205) mL/min. The loading dose was 2000 (500-3000) mg, with a maintenance dose of 2000 (500-5000) mg/day. The initial maintenance dose resulted in a predicted steady-state AUC_{24h} of 636 (253-1730) mg/L.h; 35% (45/129) of patients reached the target AUC_{24h} range, while 55% (71/129) were above and 10% (13/129) below target. After ongoing iterative dosing, 85% (110/129) eventually attained the target AUC_{24h}, with a median of 536 (401-600) mg/L.h, and this was achieved in 2 (1-12) days. In these 110 patients, the trough concentration was 13 (4-27) mg/L, and there was a poor linear correlation between AUC_{24h} and trough concentrations (R=0.42).
Conclusion. The proportion of patients achieving the target AUC_{24h} with the initial maintenance dose was low; however, this substantially improved in a short timeframe with continued dosing and monitoring. There was a poor correlation between AUC_{24h} and trough concentrations, which supports the recommendation to target AUC_{24h} directly.

544 Validated UHPLC-MS/MS assay for quantification of opioids used for cancer pain management

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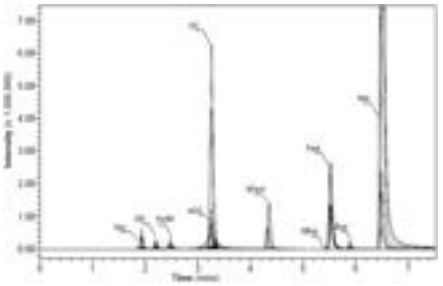

Introduction. Opioids are the gold standard for pain relief during cancer and are amongst the most common medications prescribed during palliative care. Although they are highly effective for most, some patients experience diminished analgesic efficacy or significant adverse effect profiles and there is currently no means of predicting which patients will respond best to which opioids. To begin investigating the underlying causes of these varied patient effects, we present a validated ultra-high performance liquid chromatography with tandem mass spectrometry assay (UHPLC-MS/MS) for the determination of 10 opioid compounds in human blood plasma.
Aims. To develop and validate an UHPLC-MS/MS assay and sample preparation procedure for the quantitative determination of opioids commonly used in the palliative care population of cancer patients.
Methods. Concentrations of calibrators containing all analytes were prepared based on expected plasma concentrations following clinical dosing regimens. Sample extraction was performed using protein precipitation (PPT), and analyte detection performed on an LCMS-8040 (Shimadzu, Japan).
Results. The assay demonstrated high selectivity and specificity for all opioid analytes. Recoveries for all analytes after PPT were above 90%. Matrix effects were within the assay acceptance criteria of 85-115%. QC intraday and inter-day precision and accuracy were all within assay the acceptance criteria.
Discussion. This validated assay allows for the extraction and simultaneous analysis of 10 opioid compounds using UHPLC-MS/MS and was successfully applied to plasmas of palliative care patients receiving opioids for pain management during cancer. The pharmacokinetic data gathered from this assay, in conjunction with patient genotype and clinical phenotype data could contribute to establishing a clinical opioid pharmacogenomics registry for cancer patients that would aid in personalising the prescription of opioids thus improving pain relief and reducing suffering.

545 Inhibition of human breast cancer stem cells of MDA-MB-231 and MCF-7 by phenethyl isothiocyanate from cruciferous vegetables

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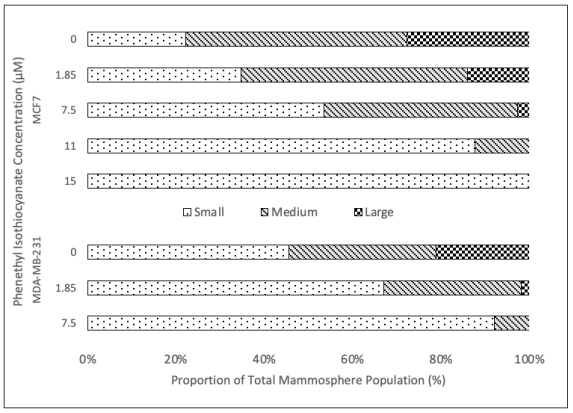
Introduction. Cruciferous vegetables are a rich source of isothiocyanates. Phenethyl isothiocyanate in particular has been shown to possess antiproliferative effects against various cancers, however there is limited information at present regarding its effects on breast cancer stem cells, a new dimension of current cancer chemotherapy.
Aims. The aim of this study was to examine whether phenethyl isothiocyanate can inhibit self-renewal and/or indeed kill the cancer stem cell population of the human breast cancer cell lines MDA-MB-231 and MCF-7.
Methods. Two main approaches were employed. They include mammospheres assays and quantitative gene expression analysis of stem marker genes using RT-qPCR.
Results and Discussion. Results indicate that phenethyl isothiocyanate reduced sphere size and number/frequency of sphere formation in a dose-dependent manner, with total inhibition of sphere formation observed at 11 µM and 22 µM in MDA-MB-231 and MCF7, respectively. Inconsistent findings were seen in the expression of stem marker genes *SOX2, vimentin,* and *ABCG2* by RT-qPCR. The results indicated that phenethyl isothiocyanate affected the expression of stem cell marker genes in a non-dose dependent manner. Expression of stem marker gene *CD44* was also reduced in the presence of phenethyl isothiocyanate.
Conclusion. In summary, overall, the findings of this study strongly support the potential role of phenethyl isothiocyanate as an anti-breast cancer therapeutic agent and/or adjunct therapy to be used together with other current anti-breast cancer drugs such us paclitaxel.



546 Using electronic prescription and laboratory data to assess prescribing practice

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Introduction. A prescription in an electronic prescribing and administration (ePA) system creates a digital data trail of a treatment decision made by a clinician. Current clinical information relevant to a prescription includes laboratory test results. Linking laboratory and prescribing data allows for investigation of the relationship between a patient's laboratory test results and prescribing decisions. Currently these data sources are not linked, and automated audit reports are constrained to single data silos.
Aims. To link electronic prescription and laboratory data. To illustrate relationships between the two datasets and use this to describe prescribing decisions.
Methods. Prescription data and laboratory test result data from 2018 to 2022 was extracted from the Canterbury District Health Board (CDHB) data warehouse. The prescription data and laboratory test result data were linked by patient, hospital admission and date/time using SQL and a report developed using PowerBI®. The report was validated using dabigatran as a test medicine, and then applied to rivaroxaban and lithium to demonstrate wider applicability.
Results. An interactive report was developed of six graphs showing the relationship of prescribing decisions to laboratory test results. Firstly, initial prescribing decisions such as choice of medicine and dose were related to laboratory test results (such as eGFR) defined in clinical guidelines. Secondly, for specific prescribed medicines, relevant laboratory results used for monitoring were compared with subsequent prescribing decisions. Prescriber decisions in response to the laboratory result were categorised as 'appropriate' or 'inappropriate' by comparing the decision with the guideline recommendations. The report links to the CDHB data warehouse, providing access to near real time information.
Discussion. Prescribing and laboratory data were successfully linked at an individual prescription and test level. Prescribing decisions such as choice of medicine and medication dose can now be compared with guideline recommendations and be rapidly audited at a systems level with minimal further resource. The proof-of-concept report is intended for use by clinical services to monitor and evaluate complex clinical decisions in near real time.

547 PBPK modeling of glimepiride in different *CYP2C9* genotypes

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Introduction. Glimepiride is a sulfonylurea antidiabetic drug. It is mainly metabolized by cytochrome P450 2C9 (CYP2C9) enzyme and produces a pharmacologically effective metabolite, cyclohexyl hydroxymethyl glimepiride. Glimepiride metabolism showed a significant difference according to the genetic polymorphism of *CYP2C9*.
Aims. Predicting pharmacokinetics by taking into account genetic polymorphisms of CYP 450 metabolic enzymes can help personalized therapeutic drug regimens. This can be realized through the physiologically based pharmacokinetic (PBPK) modeling, a method for predicting pharmacokinetics based on physiology.
Methods. PBPK model of irbesartan was developed using the PBPK modeling software PK-Sim® version 10.0. Parametric optimization was performed using the Levenberg – Marquardt algorithm implemented in PK-Sim®.
Results. The input value of each parameter for PBPK model development is shown in Table. The completed model satisfies the 2-fold-error for major pharmacokinetic parameters and especially the models which were used for development showed visual matching with the observed values in the plasma concentration profile over time. The developed model was validated with clinical data from 11 different studies on various races and doses. Most of the validation results performed through data obtained from various clinical trials were also suitable for the 2-fold error.
Discussion. The PBPK model of glimepiride in different CYP2C9 genotypes was successfully established. This model can be used for individualized pharmacotherapy

548 PBPK modeling of gliclazide in different *CYP2C9* and *CYP2C19* genotypes

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Introduction. Gliclazide is a sulfonylureas antidiabetic drug that stimulates insulin secretion in pancreatic β cells by inhibiting ATP-dependent potassium channels, that are metabolized mainly by CYP2C9 and CYP2C19. These CYP enzymes are genetically highly polymorphic, and this polymorphism can lead to different pharmacokinetic results through changes in individual metabolic capacity.
Aims. The purpose of this study is to implement the pharmacokinetic prediction of gliclazide through the physiologically based pharmacokinetic (PBPK) model according to the genotypes of *CYP2C9* and *CYP2C19*. The PBPK model, which predicts the pharmacokinetics of a drug based on physiology, can predict based on these genotype changes, which can help in individualized drug therapy.
Methods. The PBPK model was developed in the middle-out approach, and PK-Sim® (PK-Sim® 9.1, Bayer AG, Wuppertal, Germany) software was used. Physico-chemical data and ADME-related properties of gliclazide were collected from previous studies, and clinical data were measured after oral administration of gliclazide to healthy adults in different genotypes of *CYP2C9* and *CYP2C19* were used as observation data.
Results. The input value of each parameter for PBPK model development is shown in Table. The completed model satisfies the 2-fold-error for major pharmacokinetics. In addition, validation was performed by previous studies in which clinical trials on gliclazide. All of them satisfied 2-fold-error (AUC: 0.6~1.69-fold, C_{max}: 0.67~1.73-fold, $T_{1/2}$: 0.52~1.33-fold).
Discussion. In this study, the PBPK model of gliclazide was completed considering the genetic polymorphisms of *CYP2C9* and *CYP2C19* enzymes in individuals. The model was developed by reflecting the changes due to the genetic variation of each CYP metabolic enzyme well, and the developed model was reliable because of the validation with acceptable criteria.

Parameters		Input value
MW		323.40 g/mol
Log P		2.04
Fraction unbound		0.04
pK_a		4.07 & 1.38
Solubility		0.03 mg/mL
Specific Intestinal Permeability		2.12E-6 cm/s
Specific Organ Permeability		1.72E-4 cm/s
CYP2C9	V_{max}	EM: 8.934 pmol/min/pmol rec. enzyme IM: 5.130 pmol/min/pmol rec. enzyme
	K_m	EM: 384.83 μmol/L IM: 278.81 μmol/L
	Reference conc. (μmol CYP / L Liver tissue)	3.84 μmol/L
CYP2C19	V_{max}	10.088 pmol/min/pmol rec. enzyme
	K_m	304.32 μmol/L
	Reference conc. (μmol CYP / L Liver tissue)	EM: 0.76 μmol/L IM: 0.38 μmol/L PM: 0 μmol/L
Formulation	Dissolution time (80%)	3 hr

Parameters		Input value
Glimepiride		
MW		490.62 g/mol
Log P		2.82
Fraction unbound		0.50%
pKa		8.07 (acid)
Solubility		5.2 mg/L (pH 1.2) 18.4 mg/L (pH 6.8) 23.5 mg/L (pH 7.2)
Specific Intestinal Permeability		3.04E-5 cm/s
Specific Organ Permeability		2.89E-4 cm/min
*CYP2C9*1/*1*	V_{max}	7.81 pmol/min/pmol rec. enzyme
	K_m	26.87 μmol/L
*CYP2C9*1/*3*	V_{max}	4.98 pmol/min/pmol rec. enzyme
	K_m	32.80 μmol/L
*CYP2C9*3/*3*	V_{max}	1.10E-4 pmol/min/pmol rec. enzyme
	K_m	13.15 μmol/L
Formulations	Dissolution time (80%)	140 min
Cyclohexyl hydromethyl Glimepiride		
MW		506.62 g/mol
Log P		1.76
Fraction unbound		0.78%
pKa		4.32
Solubility		0.13 mg/mL
Specific Intestinal Permeability		2.58E-5 cm/min
Specific Organ Permeability		1.87E-4 cm/min
AKR1A1	V_{max}	10.98 pmol/min/pmol rec. enzyme
	K_m	9.48 μmol/L
Renal Clearance	Plasma Clearance	0.012 mL/min/kg
Bilary Clearance	Plasma Clearance	0.01 mL/min/kg

549 Immunosuppressant drug concentrations are assay-specific

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Introduction. Immunosuppressant drugs in organ transplantation are dose-adjusted to achieve target blood concentrations (KDIGO 2009). While LC-MSMS is the suggested reference method for their measurement (Brunet 2019), several immunoassays (IA) are available.
Aims. To compare concentrations of ciclosporin, tacrolimus, sirolimus and everolimus in human blood obtained by IA and LC-MSMS.
Methods. Systematic literature search of method comparison studies and extraction of linear regression slopes (IA/LC-MSMS).
Results. We identified 21, 73, 12 and 18 relevant studies for ciclosporin, tacrolimus, sirolimus and everolimus, respectively. On average, IA overestimated concentrations, but the spread of results was large (Table). Modern IA tended to perform better than older assays. The magnitude of differences in results can result in inappropriate dosing decisions.
Discussion. Performance characteristics of analytical methods need to be known for clinicians to make correct dosing decisions.

	Ciclosporin	Tacrolimus	Sirolimus	Everolimus
Mean	1.16	1.13	1.17	1.06
SD	0.19	0.14	0.19	0.14
Min	0.81	0.60	0.78	0.80
Max	1.52	1.42	1.37	1.27

KDIGO (2009) Am J Transplant 9 (Suppl 3)
Brunet M et al (2019) Ther Drug Monit 41:261–307

550 Economic evidence for deprescribing medications: a systematic review

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Introduction. Deprescribing research is an expanding area with numerous studies being conducted to assess the health outcomes of deprescribing. However, there is limited data on the economic consequences of deprescribing.
Aims. The objective of this systematic review is to appraise the economic evidence of deprescribing interventions.
Methods. A search was conducted from inception to 11 February 2019 in Embase, Medline, Scopus, DARE, PsychINFO, NHSEED, and CCTR for studies of participants of any age who had their prescribed medication(s) targeted for deprescribing and reported cost outcomes from any perspective. Study characteristics and findings were summarised qualitatively. Study quality was assessed using the Cochrane risk of bias tool and the CHEERS checklist.
Results. Of 2813 screened articles, 26 studies were included: eight aimed to reduce the number of total prescribed medications and 18 examined deprescribing a specific medication. In studies reducing the number of total medications (n=X), the direct costs of medications were compared before and after intervention in seven and to a usual care cohort in four studies, with significant reductions ranging from $8.76USD (AUD$12.93) to $40.30USD (AUD$59.47) per person per month after intervention. Cost-utility analysis was intended to be performed in 1 study where 0.05 QALY was gained by the intervention group, versus control, but not calculated due to no differences in fall-related healthcare costs between groups. In studies deprescribing specific medications classes, the most common included medications impacting the gastrointestinal system (n=11), and TNF inhibitors (n=3). Cost-utility analysis was conducted in three studies, and in one study, QALYs were gained by the intervention group versus control, deprescribing of cardiovascular medications was likely to be 70-80% cost-effective if the willingness to pay for a gain of 1 QALY was between $26 403.51USD and $39 605.26USD (AUD$38 966.30 and AUD$58 449.44).
Discussion. Preliminary results suggest there is limited, but varied, evidence that deprescribing may reduce costs. Future research considering the costs and consequences of deprescribing is needed to substantiate this evidence.

552 Prospective Study of A Clinical Quality Intervention (SMAART) to Identify and Challenge Children with "Low Risk" Penicillin and Cephalosporin Allergy

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Introduction: Beta-lactam allergies are the most common antibiotic allergies reported in children. These antibiotic allergy labels (AALs) are associated with poorer clinical outcomes and increased use of restricted antibiotics. Prior studies showed that only 5% of children admitted to hospital were evaluated for their AALs.
Aims: This study aimed to develop, implement and audit a Stratified autoMated Allergy assessment Risk Tool (SMAART) tool. The primary aim was to determine the proportion of children with AALs who were either delabelled as an inpatient or referred to the drug allergy clinic.
Methods: The tool was implemented in the electronic medical records at the Royal Children's Hospital Melbourne to risk stratify children with AAL. Children aged 0-18 years old with an AAL were included: low-risk patients were either directly delabelled based on history, offered an oral challenge test (OCT), or if they declined, referred to drug allergy clinic. Antibiotic prescribing was recorded, including the use of restricted antibiotics.
Results: During the 18-month study period, 200 admitted children had a penicillin or cephalosporin AAL. Using the SMAART tool, 180 patients were identified as low risk. Of these 180 children, 86 (48%) were referred to drug allergy clinic, 41 (23%) were delabelled after an OCT in hospital, and 40 (22%) patients were missed. The remaining 13 (7%) patients refused both an OCT and drug allergy clinic appointment.
Conclusion: To our knowledge, this is the first (EMR)-embedded (AAL) risk stratification tool for children. Using the tool, 22% of children identified as low risk using SMAART were successfully delabelled. Follow up from those children referred to drug allergy clinic is still pending.

553 Phase 1a/1b trial of a tight junction regulator (IMU-856) for coeliac disease

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Introduction. IMU-856 is an orally available and systemically acting small molecule modulator that targets an undisclosed epigenetic regulator. Preclinical studies show that IMU-856 restores barrier function and regenerates intestinal architecture while maintaining immunocompetency in the gastrointestinal tract.
Aims. To determine the safety, tolerability, and pharmacokinetics of IMU-856 in healthy volunteers.
Methods. Healthy volunteers received single ascending doses (10 to 160 mg) or multiple ascending doses (40 mg to 160 mg once daily for 14 days) of IMU-856 or placebo. Safety and tolerability were assessed by physical examination, clinical laboratory tests, vital signs, and 12-lead electrocardiograms. The concentration of IMU-856 in plasma was measured using a validated LC-MS method and pharmacokinetic parameters were estimated using non-compartmental analysis.
Results. IMU-856 showed near linear pharmacokinetics following single ascending (n=33) and multiple ascending (n= 22) doses, with minimal accumulation (C_{max} and AUC_{inf} increased ~1.5-fold after 14 days). The T_{max} was between 2 to 5 hours and the terminal t½ of IMU-856 ranged from 14-20 hours. Steady-state plasma concentrations of IMU-856 were reached after ~4 days. Most related treatment-emergent adverse events were mild in severity, with no dose-dependency. There were no clinically important findings relative to safety and tolerability.
Discussion. IMU-856 was safe and well-tolerated with a benign adverse effect profile in healthy volunteers. The pharmacokinetics of IMU-856 allow once-daily dosing. The ongoing phase 1b will provide initial data for IMU-856 in patients with well-controlled celiac disease during periods of gluten-free diet and gluten challenge.

554 Which patients are accessing medicinal cannabis under the Special Access Scheme

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Introduction. There is continuing interest in the use of medicinal cannabis products for the treatment of a wide range of indications. Sativex (nabiximols) and Epidyolex (cannabidiol) are currently the only approved medicinal cannabis products included in the Australian Register of Therapeutic Goods (ARTG). For patients to access unapproved medicinal cannabis products there is a Special Access Scheme (SAS). The TGA publishes de-identified data of SAS approvals and authorised prescribers (https://www.tga.gov.au/products/unapproved-therapeutic-goods/medicinal-cannabis-hub/medicinal-cannabis-access-pathways-and-patient-access-data).
Aims. To access the medicinal cannabis SAS approvals and authorised prescribers on the TGA website to identify the demographics and applications being used.
Methods. Extraction of data from the TGA website.
Results. The SAS-A pathway enables a prescriber to access unapproved medicinal cannabis products for an individual patient. The top 3 SAS-A indications are chronic pain, cancer pain and anxiety. Two-thirds of the SAS-A notifications are for controlled drugs under Schedule 8 (S8) of the Poisons Standard. S8 includes cannabis (including seeds, extracts, resins and the plant or any part of the plant) and tetrahydrocannabinol. The remaining third was under S4 in which the cannabidiol content is at least 98%. The median age of male patients approved to be prescribed medical cannabis under the SAS-A pathway was lower than that of female patients.
The SAS-B pathway enables a prescriber to access unapproved medicinal cannabis products for more than one patient. The number of medicinal cannabis applications approved under the SAS-B pathway was greater for males than for females. This does not reflect a greater number of patients as they may be associated with multiple approvals. For males there was 49 SAS-B applications per prescriber compared to 33 for females. The top three SAS-B applications are chronic pain, anxiety and sleep disorder. Approximately 78% of SAS-B applications were S8. The median age of male SAS-B patients was lower than that of female patients.
Discussion. The publically available SAS approval data provides insights of medicinal cannabis user demographics and applications that are being requested by medical practitioners in consultation with their patients.

555 Vancomycin AUC optimisation using minimal plasma concentration monitoring

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Introduction. The area under the plasma concentration-time curve (AUC) is recommended for guiding vancomycin dosing. It is unclear which limited sampling strategy accurately predicts the 24 h AUC at steady-state (AUC_{ss24}).
Aims. To investigate the accuracy of eight limited sampling strategies with 1-2 samples taken across a single dosing interval (e.g. C_{min}, C_{max}) on doses 1-5 of treatment for predicting vancomycin AUC_{ss24} using Bayesian forecasting, including stratification by obesity, in adults.
Methods. We performed an *in-silico* simulation study using a vancomycin PK model developed by Thomson et al (2009), implemented in the Bayesian software TCI works. We simulated virtual patients using the demographic and vancomycin dosing information from 138 patients who had been through the local therapeutic drug monitoring service. For each simulate, the estimated AUC_{ss24} for each sampling strategy was compared with the simulated reference using metrics of bias and imprecision, as well as the bioequivalence criterion for low therapeutic index medicines (0.90-1.11).
Results. The simulated patients had median (range) age 65 years (19-93), actual weight 84 kg (42-180), BMI 28.2 kg/m^2 (18.1-68.6), creatinine clearance 85 mL/min (30-302), with 98% dosed every 12 hours and median (range) simulated reference AUC_{ss24} of 618 mg/L.h (172-1726). For the whole cohort, all strategies underestimated the reference AUC_{ss24} with mean prediction error (MPE) and root mean square error (RMSE) values ranging from -71 mg/L.h (95% CI -97, -44) to -59 mg/L.h (95% CI -77, -41) and 122 to 174 mg/L.h, respectively. Only the strategy using C_{min} of doses 2 and 3 met the bioequivalence criterion. For the obese cohort (56 patients), MPE and RMSE values ranged from -103 mg/L.h (95% CI -136, -71) to -141 mg/L.h (95% CI -182, -100) and 149 to 208 mg/L.h, with no strategy meeting bioequivalence. In contrast, amongst the non-obese, all strategies met bioequivalence.
Discussion. Any of the strategies implemented could estimate AUCss 0-24h in non-obese individuals with sufficient accuracy. Further research into strategies for obese individuals is required.

Thomson AH (2009) J Antimicrob Chemother 63: 1050-1057.

556 3D printed estradiol buccal film: Design effect on film properties and drug release

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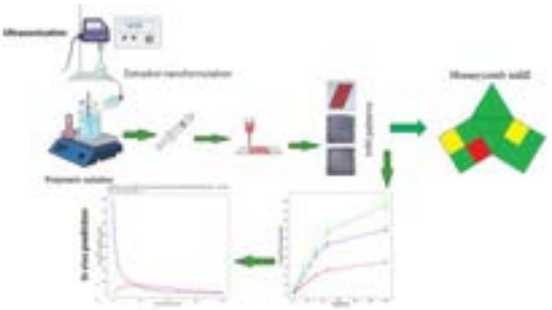

Introduction: The use of oral estrogen products for menopausal symptoms has been associated with low bioavailability and serious adverse effects such as venous thromboembolism. As a result, personalized treatment is recommended to improve efficacy and reduce adverse effects.
Aims: We aimed to employ an innovative semi-solid 3D printing technique to design and develop bi-layered estradiol film with different infill patterns.
Methods: Pressure-assisted microsyringe printing technique (Inkredible, Cellink, Gothenburg, Sweden) was used to produce the buccal films. The films were characterized for physicochemical and mechanical properties. The *in-vitro* release profile was evaluated using vertical Franz diffusion cells (PermeGear, Inc, Australia). Plasma concentration of estradiol was predicted from the *in vitro* release data using a convolution technique using R software**.**
Results**:** Films with a plain infill pattern exhibited significantly higher % elongation break and tensile strength. No interaction between components of the formulation was observed and the absence of crystallinity in the final product was confirmed by DSC and XRD analyses.The in vitro drug release study revealed the fastest drug release profile for rectangular infill (96 % within 4 hours) and the slowest drug release was observed for the plain infill pattern (~35% within 4 hours). The predicted AUC 0–4 h, C_{max}, and T_{max} were 144.85 ng·h/mL, 65.97 ng/mL, and 0.83 h for a film (honeycomb infill pattern) loaded with 1 mg of estradiol. The printing process of films with honeycomb and rectangular infill patterns was evaluated as "green" using the iGAPP tool. Discussion: The finding demonstrates that 3D printing can be used to produce films that aid personalized medicine, and the release kinetics and mechanical properties of the films can be modified by changing the infill patterns.

557 Development of a novel pH-buffered alginate gel for resistant wound infections

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Introduction. A cutaneous wound is the consequence of a tissue injury that exposes the underlying dermal tissue to pathogen invasion which often results in development of clinical infection. Wound reepithelialisation is a critical step in tissue regeneration and restoration of intact skin barriers during wound healing. A natural response to tissue injury includes a slightly acidic skin environment to prevent pathogen colonisation and changes in wound pH environment have been documented to affect the healing outcomes, however, several contradicting studies show different wound healing outcomes in response to pH changes. To identify the best pH for treating infected and non-infected wounds, novel topical pH-buffered gels were developed maintaining an acidic and alkaline wound pH environment.
Aims. To develop a novel pH-buffering formulation that maintains the wound pH acidic or alkaline following cutaneous tissue injury to promote healing in infected and non-infected wounds.
Methods. Acid and alkaline buffered gels (pH 4.5 and 7.5 respectively) were prepared using a combination of organic and inorganic acids in Milli Q water and buffered to the desired pH using a pre-standardised 1N sodium hydroxide (NaOH) solution. Xanthan gum was used as a gel base. The acid-buffering capacity of the gels was evaluated against 1N NaOH and artificial wound fluid (AWF) at different wound pH. The healing ability of the gels was determined using *in vitro* wound scratch assay and investigated for *in vitro* antibacterial activity against resistant Gram-positive and Gram-negative bacterial strains. The cell viability and rheological properties were also evaluated.
Results. Our results showed that the acid-buffered gels promote healing at pH 4.5 while alkaline-buffered gel inhibits cellular responses required for healing. The *in-vitro* antibacterial studies demonstrated inhibition for both Gram-positive and Gram-negative bacteria. The cell viability assay revealed no potential toxicity against skin cells at the antibacterial concentrations.
Discussion. Collectively, the developed pH-buffered gel indicates the potential positive role of acidic pH on cellular responses mediating tissue regeneration. Future studies are required to evaluate the antimicrobial and healing-promoting effects of the acid-buffered gel in preclinical models of wound infection.

558 Fabrication of novel hydrogels from oxidized inulin crosslinked gelatin for drug delivery

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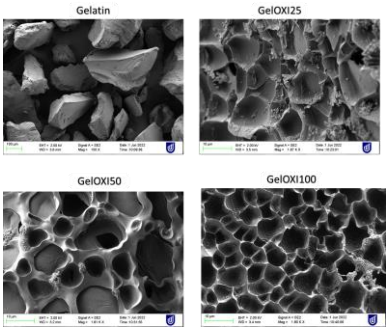

Introduction. Gelatin is commonly used for the synthesis of hydrogels because of its excellent biocompatibility, non-toxic, non-immunogenic, and biodegradability properties. However, its use has been limited by its poor mechanical and thermal stability. This drawback can be overcome by crosslinking gelatin with oxidized inulin via Schiff's base bond. Oxidized inulin synthesized by periodate oxidation was prepared and utilized as an effective crosslinker to develop gelatin hydrogel. This present study highlight how oxidized inulin can be utilized in the design and formation of stable gelatin crosslinked hydrogel.
Aims. Develop gelatin hydrogel with better physicochemical properties suitable for drug delivery application.
Methods. Inulin was modified to obtain a promising crosslinking agent using a previously reported method. Gelatin hydrogels were prepared by crosslinking gelatin with oxidized inulin. Briefly, gelatin was dissolved in 2ml of water at 60 ℃ with continuous stirring and oxidized inulin was dissolved in 2ml of 0.01M Borax solution. The resulting solution was mixed and allowed to crosslink resulting in the formation of a hydrogel. Three different hydrogel batches were prepared using a different mass ratio of oxidized inulin in order to elucidate the effect of the relative ratio of inulin on the hydrogel properties (Figure 1). The prepared hydrogels were characterized for their physicochemical properties
Results. Both ^{1}HNMR and ^{13}C NMR confirmed the formation of the aldehyde group in oxidized inulin. In addition, the FTIR analysis result complements the NMR result with a peak around 876 and 1725 cm^{-1} confirming the formation of the aldehyde group. FTIR also confirms the crosslinking of gelatin with oxidized inulin. The crosslinking was also further confirmed by DSC. SEM clearly shows that hydrogels are highly porous materials. The use of a varying ratio of oxidized inulin results in hydrogels with different pore sizes.
Discussion. The finding illustrates oxidized inulin can be effectively utilized as a crosslinking agent and the developed gelatin hydrogel can potentially be used to deliver drugs

559 Triton X-100 alternative surfactants for split-virus influenza vaccine

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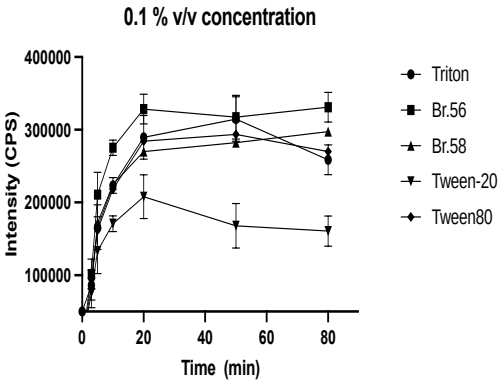
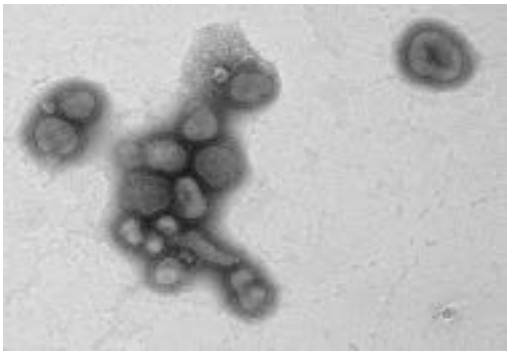


Introduction. Vaccination is the best approach to prevent influenza. Inactivated virus vaccines are the most common influenza vaccines due to their proven safety profiles and relatively low production costs. Generally, a non-ionic surfactant such as Triton X-100 is used to split influenza virus when preparing inactivated vaccines. However, Triton X-100 was listed as "substance of very high concern" by the European Commission and hence it has been used only for exceptional circumstances since January 2021. Prospective alternative must not include the phenyl ether moiety of Triton x-100 to prevent the formation of degradation products that could mimic estrogen.
Aims. Finding an alternative surfactant for Triton X-100 in splitting viruses with comparable physicochemical features, including solubility, ease of removal, and non-degradation to hazardous metabolites (eco-friendly) that can be used in the manufacturing of inactivated vaccines.
Methods. We used a variety of different techniques to study surfactant–virus interactions and elucidate their ability to split the virus, including but not limited to fluorescence and light scattering (NanoSight and dynamic light scattering) spectroscopy, and transmission electron microscope. We have tested these surfactants on different influenza virus strains including A/Switzerland/9715293/2013 (H3N2) and B/Phuket/3073/2013 (H3N2).
Results. Our results show that Tween-20, Tween-80 and Brij-58 are able to split the virus at high concentrations. However, Brij-56 show a comparable effect to Triton X-100, and it is able to split the influenza virus within the same concentration range to Triton X-100.



560 Pharmacokinetics of Bee Venom Phospholipase A2 in rats using a sandwich ELISA

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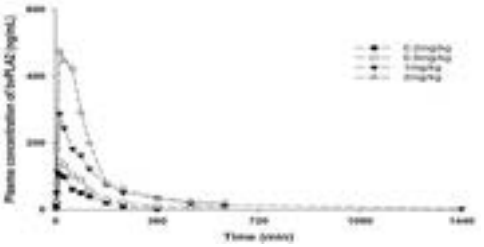

Introduction. Alzheimer's disease is the most common form of dementia and 75% of dementia patients have Alzheimer's disease. In one experiment, test mice with Alzheimer's disease were injected with the amyloid beta peptide antigen and the bee venom phospholipase A2 (bvPLA2). component of bee venom, and cognitive and memory were measured through a Morris water maze test. As a result of this experiment, there is a study that the rats injected with bvPLA2 had a remarkable increase in cognitive function close to that of normal rats compared to the group injected with only the amyloid vaccine. European honeybee contains about 10%-12% of bvPLA2. The new composition of bee venom (NCBV) is a substance with a high content of bvPLA2 (75% or more) than that of natural bee venom. Currently, a treatment for Alzheimer's disease using NCBV is being developed in Korea.
Aims. To evaluate the pharmacokinetic profiles of bvPLA2 after subcutaneous injection of NCBV in male and female rats.
Methods. We did pharmacokinetic studies of bvPLA2 after single subcutaneous injection of NCBV at doses of 0.2, 0.5, 1, 2 mg/kg to male and female rats. Serial blood sampling was collected and time points ranged from 0 to 1440 min. To analyze the plasma levels of bvpla2 our developed a sandwich ELISA was used, with LLOQ of 0.78 ng/mL.
Results. Pharmacokinetic parameters, such as The C_{max} and AUC values of bvPLA2 of male were approximately 2 times higher than female rats. The C_{max} and AUC values of bvPLA2 displayed linear pharmacokinetics with doses ranging from 0.2 mg/kg to 2 mg/kg, with no unexpected accumulation.
Discussion. This study to characterize the pharmacokinetic properties of bvPLA2 in NCBV after subcutaneous injection of NCBV in male and female rats.

562 Development of PBPK models to predict the effect of rabeprazole on vismodegib

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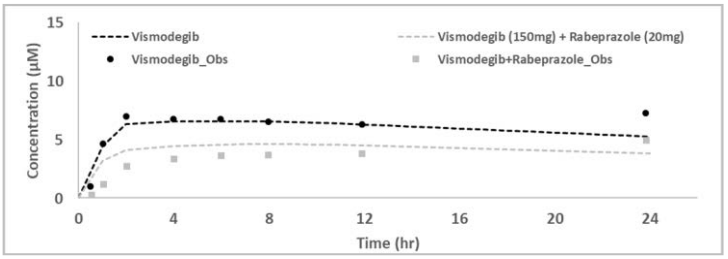

Introduction: Vismodegib is a weakly basic drug exhibiting unique nonlinear oral absorption properties related to the poor and pH-dependent solubility. Therefore, the coadministration with drugs that elevate gastric pH such as acid-reducing agents (ARAs) might lead to differences in the solubility of stomach and result in the pharmacokinetic (PK) profile of vismodegib. Advanced compartmental absorption and transit (ACAT) model is a useful tool for predicting the PK profile changes due to alteration of gastric environment.
Aims: To develop the physiological based pharmacokinetic (PBPK) model of vismodegib reflecting nonlinear and pH-dependent absorption.
Methods: We did comprehensive search of literature and collected PK profile data and changes with ARAs of vismodegib (Vikram et al., 2016). Data from the PK study were extracted via WebPlotDigitizer (WebPlotDigitizer, Version 4.5, Ankit Rohatgi). Mrgsolve as an R package was used to build PBPK model of vismodegib. Predictive performance was considered successful if predicted and observed PK parameters were within a 2-fold error ratio.
Results: Pharmacokinetic parameters such as AUC, C_{max} decrease after co-administered with rabeprazole. The simulation data were fit suitable criteria in both single and multiple dose simulation.
Discussion: PBPK model of vismodegib predicted well that there was a limited interaction with rabeprazole in the case of multiple dose administration rather than single dose administration.

Vikram et al. (2016) Cancer Chemother Pharmacol 78(1):41-9

563 Measurement of P_{app} using HiPSC-IECs and prediction pharmacokinetic profiles using PBPK models

Chae Bin Lee[1], Soon Uk Chae[1], Seongjun Jo[1], Sangyoung Lee[1], Sabin Shin[1], Soo Kyung Bae[1]. College of Pharmacy, The Catholic University of Korea[1], Bucheon-si, Gyeonggi-do, Republic of Korea.

Introduction: There has been always demands for development of orally administered drugs majorly due to convenience and safety. For orally administered drugs, they are highly exposed to gastrointestinal metabolism and transport system. However, in vitro tools to predict gastrointestinal pharmacokinetic properties are limited. These days, each organic cell lines induced from human induced pluripotent stem cells have been developed and it is noticed as useful tools to alternate primary tissue cell.
Aims: This study aims to show human induced pluripotent stem cell derived intestinal epithelial cells (HiPSC-IECs) can be useful tool to predict pharmacokinetic profiles of orally administered drugs.
Methods: Apparent permeability of 22 test drugs (including high permeability drugs, CYP3A4 probe drugs and negative control for permeability and intestinal metabolism) were measured using HiPSC-IECs on 24 trans-well after 7 days of incubation. Calculated P_{app} value is used to predict human effective permeability from correlation with human absorbed fraction. Test drugs were grouped with F_a and F_g values (Criteria for F_a: 0.85, Criteria for F_g: 0.66). Using predicted P_{eff} clinical pharmacokinetic properties were predicted using physiologically based pharmacokinetic model using simCYP™ simulator.
Results: Simulated concentration profiles of test drugs in each group are comparable to observed data from literature. The pharmacokinetic parameters including $AUC_{0\text{-}t}$, C_{max}, and T_{max} are also comparable to observed data.
Discussion: HiPSC-IECs might be a useful tool to predict clinical pharmacokinetic properties of orally administered drugs.

564 Restoring multi-drug resistant Gram negative bacterial susceptibility to Fosfomycin

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Introduction. Antimicrobial resistance is estimated to be the cause of more than 700,000 deaths worldwide and this number is predicted to rise to 10 million by 2050 (O'Neill, 2014). Fosfomycin is a broad-spectrum antibiotic with high bioavailability and low toxicity but in recent years, resistance to it has increased in Gram negative bacteria, with the drug-modifying enzyme, FosA, being a leading cause.
Aim. This study aims to find a potent FosA inhibitor to be given as an adjuvant, thus re-establishing fosfomycin susceptibility in multi-drug resistant Gram negative pathogens.
Methods. *In silico* screening was conducted using the FosA crystal structures extracted from *Klebsiella pneumoniae* ($FosA^{KP}$), *Escherichia coli* (FosA3) and *Pseudomonas aeruginosa* ($FosA^{PA}$). Structure Based and Ligand Based Drug Design, using Maestro Schrödinger, involved scaffolds based on the known inhibitors ANY1 and phosphonoformate (PPF). The compounds with the best docking and fitness scores were selected for antibacterial activity testing, which was conducted using broth microdilution.
Results. 590,000 compounds were initially screened (including approved drugs and compounds generally regarded as safe) to identify compounds with docking scores greater than -8 $kCal.mol^{-1}$. We will discuss the results from the microbiological testing in the poster/presentation.
Discussion. The compounds with the highest binding scores contain charged and polarised groups, leading to the formation of unique ion-ion, ion-dipole and pi-pi interactions with FosA residues and significant binding to the central Mn^{2+} ion. All the selected compounds were predicted to bind more effectively than fosfomycin to $FosA^{KP}$, reinforcing their potential to competitively inhibit the enzyme. The *in vitro* activity of these compounds will be confirmed with laboratory antibacterial testing.

O'Neill, J. (2014). *Antimicrobial Resistance: Talking a crisis for the health and wealth of nations.*

565 Economic and Time-Efficient Method for API Extraction from Equine Pharmaceutical Formulations

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Introduction. PIDG has developed an oral paste containing Pergolide Mesylate. Analytical methods were required to assist the formulation optimization, and to evaluate its stability. However, literature methods were not applicable to this API. Samples with low concentrations of analytes are well-established as a major obstacle in analytical chemistry. In addition to this, there is a driving force toward a less time-consuming drug development process, enabling a more economic method which reduces solvent and chemical consumption.
Aims. To establish and validate a reliable HPLC method for the routine characterization of Pergolide Mesylate formulated as a paste.
Methods. Two different methods for the extraction of Pergolide Mesylate from the paste were trialled. The first approach involved the storage tubes containing the prepared formulation, extracted through a mixture of solvents, and homogenised by a commonly used laboratory vortex at 1500 RPM. In the second method, the extracting solutions were homogenised by using a Multi Tube Vortex Mixer (as per Figure above), at the highest speed of 3000 RPM.
Results. The regression lines (of the calibration curve) were found linear for both methods. In addition to this, an average recovery of 99.8±1.3 % for Pergolide Mesylate was obtained using the two described methods. Most interestingly, in the latter approach involving the Multi Tube Vortex Mixer, a much lower quantity of sample and solvent was used, up to 50% of sample amount and 25% of solvents respectively. Most importantly, with this approach, the analysis time was significantly reduced by almost 6-fold, from 2 hours to 25 minutes.
Discussion. Based on the validated data, an economic, greener, and timely analytical method was successfully developed, allowing quantification of samples with low analyte concentration.
Ali Sarafraz Yazdi (2011) TrAC Trends in Analytical Chemistry 30:918-929

566 Development of remdesivir and ebselen containing inhalable dry powder for COVID-19

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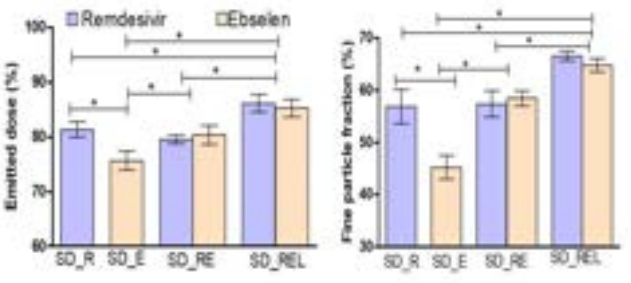

Introduction. Respiratory drug delivery is considered the most logical approach to treat COVID-19, as the respiratory tract is the main anatomical site of replication of SARS-CoV-2. *In vitro* studies have shown that the U.S. FDA-approved antiviral agent remdesivir can synergistically inhibit SARS-CoV-2 replication when combined with ebselen, a protease inhibitor [1]. Thus, delivering these drugs directly to the target site as a combination may ensure a better therapeutic outcome than the individual agents.
Aims. The current study aimed to develop and characterize the inhalable dry powder of remdesivir and ebselen.
Methods. The dry powder of remdesivir (SD_R), ebselen (SD_E), and their combination (in the presence/absence of leucine: SD_REL and SD_RE) were prepared by the spray-drying technique with the fixed feed concentration (0.8% $^{w}/_{v}$) in methanol. SD_RE consisted of remdesivir and ebselen in 1:1 molar ratio and SD_REL consisted of remdesivir, ebselen and leucine in 1:1:1 molar ratios.
Results. The prepared dry powders were spherical in shape and within the size range of 1–3 µm. All the dry powders except for the SD_R were crystalline. SD_R was amorphous in nature. The emitted dose (ED) and fine particle fraction (FPF) of SD_R were 81% and 56% whereas these values were 75% and 45% for SD_E. The ED and FPF were found 80% and 57% for SD_RE. A higher ED (>86%) and FPF (>67%) were obtained for SD_REL. All the dry powders had <1.5% ($^{w}/_{w}$) water content and were non-toxic to the cultured human airway epithelial cells (Calu-3) up to 100 µM.
Discussion. The non-toxic combinational dry powder containing remdesivir and ebselen was developed successfully by the spray-drying technique. Further *in vitro*, animal model studies will test the ability of these formulations to inhibit SARS-CoV-2. 1. Chen T et al. (2021) ACS Pharmacol Transl Sci. 42: 898–907.

567 Development of LC-MS/MS Analysis for Pergolide in Equine Plasma

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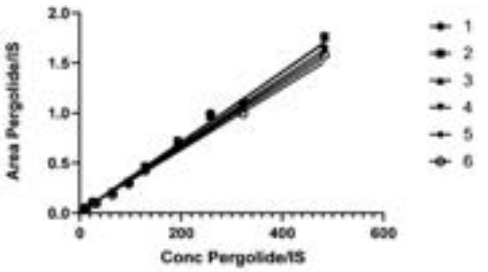

Introduction. An LC-MS/MS method was developed to quantify pergolide in the plasma at picogram (pg) per milliliter level. High sensitivity is required to fully characterize the pharmacokinetics (PK) profiles of the pergolide formulations
in plasma.
Aims. Use LC-MS/MS and develop an analytical method to quantify pergolide (pg/mL) in equine plasma.
Methods. Sample preparation process: 400 µL of plasma was placed into a 1.5 ml Eppendorf tube; spiked with 20 µL IS; incubated at room temperature for 30 minutes in darkness. Protein Precipitation process: 820 µL of ice-cold methanol was added to the spiked plasma; vortexed for 1 minute, and put in a freeze for 30 mins; centrifuged 20 min at 16,000 rcf at 5°C. The supernatant was then removed and placed into an HPLC vial for LC-MS/MS injection and analysis.
Results. The blank plasma sample's chromatogram displayed minimal interference with a small peak which was below the detection level for pergolide, the developed method has required selectivity for pergolide. The developed analytical method achieved an LLOQ of 6.45 pg/mL with great accuracy. The linearity (6.45-483.87 pg/mL) has good repeatability. Intraday precision of the peak area was very good across the range of required concentrations.
Discussion. The developed and validated LC-MS/MS method could achieve an accurate assay for pergolide formulations' PK studies in horses.

568 5-fluorouracil and leucovorin predictive pharmacokinetics after administration of standard treatment and Deflexifol™

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Introduction. 5-fluorouracil (5-FU), in combination with its biomodulator leucovorin, remains a fundamental component of many efficacious chemotherapy regimens for patients with solid tumours. Due to physical incompatibility issues, standard administration schedules involve sequential administration of leucovorin, followed by 5-FU. Given the importance of leucovorin in modulating 5-FU activity, simultaneous administration of 5-FU and leucovorin is expected to enhance anti-tumour effect. To address this potential, Deflexifol™ has been developed as an all-in-one injectable reformulation of 5-FU and leucovorin at physiological pH.
Aims. To examine the comparative pharmacokinetics of 5-FU and leucovorin after administration of US/EU and AU standard treatment regimens, and Deflexifol™.
Methods. The pharmacokinetics of 5-FU, racemic leucovorin and L-leucovorin (active enantiomer) after administration of standard treatment with infusional leucovorin (US/EU regimen), standard treatment with bolus leucovorin (AU regimen), and Deflexifol™ treatment were compared using a pharmacokinetic modelling and simulation approach.
Results. 5-FU, racemic leucovorin and L-leucovorin were best described by two-compartment models, with first-order elimination from the central component. Model diagnostic plots indicated uniform distribution and a lack of bias, and external validation confirmed model suitability. For racemic leucovorin, simulations indicated that Deflexifol™ is expected to have an equivalent or longer period of concurrent 5-FU and leucovorin exposure, compared to standard treatment. Most importantly, when considering the pharmacologically active L-enantiomer of leucovorin, synergistic action of 5-FU and leucovorin is predicted to occur over 46.7 hours for Deflexifol™, compared with only 3.42 hours and 1.76 hours for standard infusional (US/EU) and bolus (AU) regimens, respectively.
Discussion. This work provides preliminary evidence to support the enhanced duration and extent of leucovorin exposure with Deflexifol™ treatment. Based on this, it is anticipated that Deflexifol™ treatment would maximise clinical activity and enhance anti-tumour effect when compared to current treatment regimens.

569 Palmitoylethanolamide (palmidrol) as an analgesic and anti-inflammatory treatment for diabetic-related peripheral neuropathy

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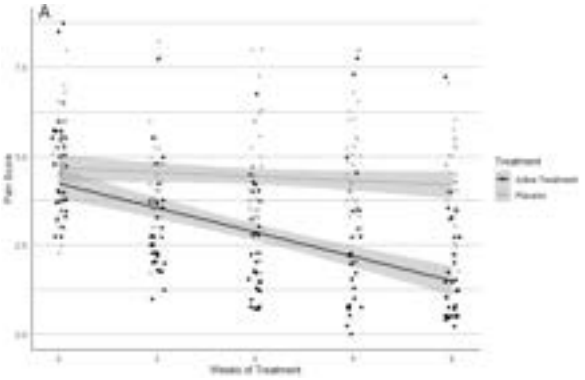

Introduction. Diabetic peripheral neuropathy (DPN) is a common complication of diabetes, potentially leading to amputation. Treatment can be difficult, often requiring increasing pain medication which can have side effects. Palmitoylethanolamide (PEA or palmidrol) may be a potential safe treatment for DPN.
Aims. To assess efficacy of a palmidrol supplement (Levagen+™) for pain and inflammation in those with DPN and taking diabetes medication (metformin and/or insulin).
Methods. A double-blind randomised clinical trial of 70 adults diagnosed with DPN and taking diabetic medication took 600 mg of palmidrol or placebo daily for 8 weeks. Total pain score (PS) and pain interference (PI) were measured with the BPI-DPN. Inflammation was measured as interleukin-6 (IL-6) and c-reactive protein (CRP).
Results. By week 8 total PS and PI levels significantly reduced in the palmidrol group (both $p<0.001$), as well as IL-6 and CRP (both $p=0.05$). The treatment was well tolerated. There was no change to blood sugar levels in both groups.
Discussion. Previous studies have found palmidrol to be a well-tolerated analgesic with anti-inflammatory action. This study supports the use of palmidrol as an analgesic and anti-inflammatory treatment in those with DPN and as an adjunct to diabetic-related medication.

570 Pharmacokinetic-pharmacodynamic modeling and simulation in the dosing of methotrexate.

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Introduction. Methotrexate (MTX) is effective in controlling disease activity in rheumatoid arthritis (RA). The therapeutic effect of MTX is mediated by intracellular methotrexate polyglutamates (MTX-PG). However, responses to MTX are highly variable, which complicates the process of individualising MTX dose regimens.
Aims. To develop a non-linear mixed effect (NLME) PK model to describe the MTX-PG concentration in erythrocytes (ery-MTX-PG) and linking the ery-MTX-PG concentration (PK) to the 28-joint Disease Activity Score (DAS28) (PD).
Methods. Data from the study (Sandhu et al, 2017) with 117 RA patients were used to develop the model. The estimation of population PK parameters was performed using the stochastic approximation expectation maximisation (SAEM) algorithm in Monolix 2020R1. The model was used to simulate the varying dosing regimens and the simulations were applied to 1000-virtual-patient data set using R version4.2.1. A mixed effect linear regression model was used to describe the relationship between DAS28 and ery-MTX-PG concentration. The ery-MTXPG-concentration-time and DAS28-time series plots were simulated for 24 weeks following an MTX loading dose regimen, consisting of 3X50mg weekly SC MTX doses, followed by 20mg weekly oral MTX. This was compared to a standard dose regimen.
Results. Ery-MTX triglutamate (ery-MTX-PG_3) had the best goodness-of-fit plot as described by the PK model, and its concentration was best described by a one-compartment model with first-order absorption and linear elimination. The model also included patient age, ery-MTX-PG_3 concentration and use of prednisolone in the first 4 weeks. The concentration-time profile and the DAS28-time plot showed high interpatient variability. With a loading dose, mean ery-MTX-PG_3 concentration peaked at 154.4±1.1 nmol/L (n=1000) at 2.5 weeks, before reducing to the trough concentration 53.9±0.4 nmol/L (n=1000) at 24 weeks. Higher ery-MTX-PG_3 concentration correlated to more reduction in DAS28.
Discussion. A loading dose regimen was more likely to achieve higher ery-MTX-PG_3, concentration and a larger reduction in DAS28.
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571 Transcriptome analysis of rapidly maturing CLEFF4 and age matched parent Caco2 cells

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Introduction. The CLEFF4 sub clone from stock Caco2 cells has a unique property of being able to develop polarised cell monolayers with high P-glycoprotein (P-gp)/(ABCB1) expression and tight junctions much quicker than the original cell line. Instead of being useful for transport studies 21-24 days after initiating culture, the CLEFF4 cell line matures in 5-6 days with tight junctions surpassing that of 3 week old Caco2 cells in that time frame. This has enabled the CLEFF4 cell line to provide measures of permeability, which predicts oral absorption for potential drug candidates, so important for pre-clinical drug development, 4 times faster than the original cell line. Aim. RNA samples were collected and analysed at day 4 and day 7 of culture and had complete RNA transcriptome analysed by Genomics WA followed by quantitative analysis by the ranaseq.eu open bioinformatics platform. Results. Differential expression data from the FASTQ files have shown significant differences in mRNA (in Transcripts per million copies [TPM]) from a key tight junction protein as well as P-gp, which matches the increases shown in earlier work from the laboratory, and a range of other gene expressions, including key phase 2 metabolism enzymes and other factors that may hold the key to understanding accelerated human cell maturation. Conclusion. These gene expression results may be significant for other tissues beyond the gastrointestinal tract, and potentially for accelerated cell growth for the new field of laboratory grown tissues for organ replacement.