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Biomedical & Health Sciences

abs #002

Validation of an Automated System for Detection of Dental Caries using 3D Models of Primary Teeth

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Background. An automated caries scoring system (ACSS) using light-induced fluorescence has been developed for intraoral scanners to support early identification of dental caries (‘decay’) prior to cavity formation. The ACSS has been validated on permanent teeth, and in vitro for primary teeth. This study investigated the in vivo validity of the ACSS for detection and classification of occlusal carious lesions in primary teeth compared to visual examination (VE).

Methods/Results. A sample of 5-year-old healthy children (n=216) underwent VE and caries experience was recorded using the International Caries Detection and Assessment System (ICDAS). Scanning took place using the TRIOS4 scanner to produce 3D models. The ACSS was applied to primary molar occlusal surfaces on the 3D models using the Trios Patient Monitoring software (v2.3, 3Shape-TRIOS-A/S-Denmark). Surfaces were classified as sound, initial caries (ICDAS 01/02) or moderate/extensive caries (ICDAS >03). The performance of the ACSS was compared to VE using logistic regression with generalised linear mixed models. All primary molar occlusal surfaces (n=1660) were assessed with the ACSS, leaving n=1607 eligible for analysis after exclusion of restored teeth (n=53). Compared to VE, the odds of detecting caries using the ACSS were 30% lower at the initial disease threshold (OR 0.70, 95% CI 0.50-0.96), and 69% lower at the moderate/extensive disease threshold (OR 0.31, 95% CI 0.16-0.62).

Conclusion. The findings suggest that the ACSS and VE do not show good agreement. The ACSS could be adjusted for use in primary teeth by validating the scoring cut-offs against histological reference standards on primary teeth.

abs #006

Improving the iNTEGration between pRimary And inTensive carE after critical illness (INTEGRATE) study

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Background. Critical care survivors experience multiple care transitions (Mikkelsen et al. 2020 CCM,Needham et al. 2012 CCM). Internationally, there is limited specialised Intensive Care Unit (ICU) follow-up service data for these patients3. Given the relative ubiquitous nature of primary care, general practitioners (GPs) are ideally positioned to provide support post-hospital, however are rarely included in post-ICU care research (Admon et al. 2019 Lancet Respiratory Medicine). The aim of this study was to identify unmet care needs and solutions to improve the coordination and integration of care following an ICU admission – from the perspective of patients, their caregivers, intensivists, and GPs.

Methods/Results. Qualitative study using the Framework Analysis Method, reported using the COREQ (Tong et al. 2007 Int J Qual Health Care) checklist. Participants were ICU patients, their caregivers and intensivists, recruited from three academic hospitals; and GPs, recruited from a state-wide, academic-affiliated network. Purposive sampling was used to achieve socio-economic diversity (using the Australian Bureau of Statistics Index for Relative Socioeconomic Advantage and Disadvantage), and differing survivorship experiences. Individual semi-structured interviews were completed, and patient demographic information was collected from medical records. Qualitative data were audio-recorded, transcribed, and coded independently by two experienced researchers to synthesise and generate themes.

Forty-six interviews (15 patients, 8 caregivers, 15 intensivists, and 8 GPs) were conducted between 6 and 12 months post ICU discharge. The majority of patients were originally admitted to ICU for cardiac or respiratory failure, or COVID-19 diagnoses, and received mechanical ventilation.

Within these feedback loops, eight major themes were identified and categorised. Feedback loop 1: Increased linkages and collaboration between ICU and GP, ICU and GP clinician education on screening for post ICU issues and the role of ICU respectively. Feedback loop 2: Improved survivorship focussed communication from ICU to GP utilising digital health, Role of allied health to support across the recovery care arc, Patient information portal; and Feedback loop 3: Comprehensive post-ICU care pathway model akin to existing cancer pathways, Improved awareness and accessibility to community support including virtual rehabilitation, and the Role of ICU follow-up for clinician morale and contribution to ICU care through awareness of critical care outcomes.

A conceptual model was identified as foci for research and service improvement initiatives: Feedback loop 1: Between GP and ICU; Feedback loop 2: ICU to GP; and Feedback loop 3: ICU follow-up programs back to the ICU.

Conclusion. Eight major themes to improve enhanced recovery support for critical care survivors within existing healthcare system structures by leveraging existing resources and expertise were identified. Our data highlights the importance of comprehensive communication, active relationships between primary and intensive care clinicians, and the value of allied health in managing this cohort. These themes are mapped to a novel conceptual model that includes key feedback loops for health system improvements and foci for future interventional trials to improve ICU survivorship outcomes.

abs #022

Development of a self-directed online Tai Chi intervention for people with knee osteoarthritis

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Background. Knee osteoarthritis (OA) is a leading contributor to global disability. While evidence supports the effectiveness of Tai Chi for improving symptoms for individuals with OA, access to in-person Tai Chi classes may be difficult for many people due to logistical constraints and cost. To overcome accessibility barriers, a self-directed online Tai Chi intervention for OA is needed.

Methods/Results. A self-directed online Tai Chi intervention for OA was developed iteratively. First a panel of Tai Chi instructors and consumers with OA was assembled. A survey was then completed by the panel and other participants to rate 32 Tai Chi movements on their appropriateness, safety, and practicality for the program. The second survey, using conjoint analysis methodology, asked participants to prioritize Tai Chi movements through pairwise comparisons. Based on the results and a panel group discussion, a Tai Chi program was developed and rated by the panel. The program was housed within a customized “MyJoint Tai Chi” website with its usability assessed through online think-aloud sessions with OA participants.

The first two surveys (n=35, n=27 respectively) identified a ranked list of 24 Tai Chi movements for potential inclusion. The Yang style 10 forms, with modifications, received 92% agreement from the final panel survey (n=13) and was used as the core of the final program. The "MyJoint Tai Chi" website prototype was refined based on user feedback (n=5).

Conclusion. This study presents the development of a self-directed online Tai Chi intervention (“MyJoint Tai Chi”). Its effectiveness will be evaluated in a planned randomized control trial.

abs #047

Back to the CD4+ T cell: a promising non-invasive immune diagnostic for coeliac disease

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Background. Coeliac disease (CD) is a common illness where dietary gluten triggers proinflammatory CD4+ T cells. Diagnosis remains suboptimal, compounded by the requirement for an invasive gastroscopy and active gluten intake. We showed that rare gluten-specific CD4+ T cells can be indirectly detected by measuring interleukin (IL)-2 release acutely following oral gluten-challenge in coeliac patients following a gluten-free diet (GFD) and leveraged this finding to develop a whole blood IL-2 release assay (IL2-RA).

Methods/Results. Adult participants with treated CD (GFD), untreated CD (gluten-containing diet), non-coeliac gluten sensitivity (NCGS), healthy controls (HC) and non-CD autoimmune disease controls (AIDC) were recruited. Fresh whole blood was incubated for 24 hours with gluten peptides and IL-2 release assessed (MSD). We assessed the IL2-RA in 55 treated CD, 2 untreated CD, 9 NCGS, 30 HC and 4 AIDC and showed 86% sensitivity (48/54) and 92% specificity (42/43) to detect CD. HLA-DQ blocking (SPVL3) significantly reduced IL2-RA. IL2-RA was performed weekly for 4 weeks after untreated CD started a GFD and found the IL2-RA significantly increased after 2 weeks on GFD then plateaued. We performed single dose gluten-challenges in 10 treated CD to compare in vivo circulating IL-2 release after 4 hours with ex vivo IL2-RA and found these metrics were consistent.

Conclusion. The IL2-RA is highly sensitive and specific for CD, comparable to existing CD serology with the added benefit of detecting treated CD on a GFD and is a useful tool for immunomonitoring in clinical trials.

abs #054

5Z-7-oxozeanol is protective against fibrotic phenotypes in cultures of human Tenon's fibroblasts

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Background. Glaucoma is a growing health concern, and 1 in 50 Australians will develop the condition. Glaucoma filtration surgery (GFS) is the most effective treatment method, but it can activate Tenon's fibroblasts (HTFs) which cause ocular fibrosis. Transforming growth factor β (TGFβ) is a profibrotic growth factor that plays a major role in ocular fibrosis. TGFβ activated kinase-1 (TAK1) is a kinase that regulates TGFβ signaling, and is key in fibrosis pathogenesis. This study intends to demonstrate the protective antifibrotic effects of inhibiting TAK1 activity using 5Z-7-oxozeanol in HTFs.

Methods/Results. HTFs cultured from GFS patients were used for cell viability, cell proliferation, gel contractility and ELISA bioassays. In all experiments, three conditions were tested to investigate the effect of 5Z-7-oxozeanol.: (1) media alone, (2) TGFβ1 (10 ng/mL) + vehicle, or (3) TGFβ1 (10 ng/mL) + 5Z-7-oxozeanol (0.1 to 30 uM). Cell-TeterGlo assessed cell viability and proliferation, collagen gel contraction assessed the contractile ability of HTFs in a three-dimensional collagen matrix, and ELISA detected the protein expression of fibrotic markers.
5Z-7-oxozeanol did not show toxicity up to 10uM ($P<0.001$), thus it was tested up to 10uM in all assays. 5Z-7-oxozeanol reduced TGFβ1-induced HTFs proliferation ($P<0.05$). Comparative measurement of gel area showed that 5Z-7-oxozeanol also suppressed TGFβ1-induced cell contractility ($P<0.05$). The expression of a-SMA, fibronectin and collagen were also significantly lower with 5Z-7-oxozeanol treatment ($P<0.001$).

Conclusion. 5Z-7-Oxozeaenol can inhibit the profibrotic effects of TGFβ1 on HTFs, highlighting the feasibility of targeting TAK1 activity as a strategy to alleviate fibrosis resulting from GFS.

abs #055

A novel meibomian glands automatic segmentation model in infrared images with limited annotation

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Background. To explore a novel meibomian glands segmentation framework only with limited annotation for alleviating the burden on ophthalmologists and enhancing the efficiency of clinical diagnosis.

Methods/Results. METHODS: 203 infrared meibomian images from 138 patients with dry eye disease, along with corresponding annotations, were collected for study. A rectified scribble-supervised glands segmentation (RSSGS) model including temporal ensemble prediction, uncertainty estimation, and transformation consistency constraints was proposed to address the limited supervision information resulting from the scribble annotation. The feasibility and effectiveness of the proposed model were analysed by accuracy, intersection over union and dice coefficient.
RESULTS: Taking manual label as the gold standard, RSSGS achieved impressive results with accuracy of 93.54%, dice coefficient of 78.02%, and intersection over Union of 64.18%. Notably, these performance metrics surpass the current state-of-the-art weakly supervised methods by 0.76%, 2.06%, and 2.69%, respectively. Moreover, with about 80% reduction in annotation cost, it is only 0.72%, 1.51%, and 2.04% lower than the fully annotated methods.

Conclusion. We have developed a novel automatic segmentation model for meibomian glands in infrared eyelid images, employing scribble annotation for training. Remarkably, it maintains an exceptionally high segmentation accuracy while significantly reducing the training costs. It would be quite useful for clinical parameters calculation, greatly improving the diagnostic efficiency of ophthalmologists in evaluating meibomian gland dysfunction.

abs #064

Cerebral haemodynamics and orthostatic response to upright position in acute ischaemic stroke: the CHORUS study

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Background. The effects of upright positions (sitting, standing) on cerebral haemodynamics in acute ischaemic stroke are not well understood. A potential mechanism for harm is that upright positioning could worsen cerebral perfusion early post-ischaemic stroke.

Methods/Results. We recruited ischaemic stroke (<48h onset) participants with and without occlusive disease, and controls to investigate the effects of upright positions on cerebral haemodynamics. We investigated MCA mean velocity (MV) using transcranial Doppler in 0° head position then 30°, 70°, 90° sitting, and 90° standing (<48h and 3-7days post-stroke). A blinded assessor determined MV. Mixed effect linear regression was used to analyse changes in MV from 0° to other head positions. Logistic regression was used to explore associations between MV and 30-day functional outcome.
Forty-two stroke participants (13 with occlusive disease, 29 without) were recruited. Affected hemisphere MV decreased in stroke with occlusive disease (<48h): from 0° to sitting (-9.9cm/s, 95%CI [-16.4,-3.4]) and standing (-7.1cm/s, 95%CI [-14.3,-0.01]); and in stroke without occlusive disease from 0° to sitting (-3.3cm/s, 95%CI [-5.6,-1.1]) and standing (-3.6cm/s, 95%CI [-5.9,-1.3]) (p-value interaction=0.07). Similar changes were observed in controls (0° to sitting -3.8cm/s, 95%CI [-6.0,-1.63] and standing -3cm/s, 95%CI [-5.2,-0.81]) (no significant interaction stroke vs controls). Orthostatic changes MV in stroke <48h were similar to 3-7 days. No association between orthostatic changes in MV <48h and 30-day functional outcome was found.

Conclusion. Moving to more upright positions in the first 2 days post-stroke does reduce MV, but these changes were not significantly different for people with stroke with and without occlusive disease.

abs #067

T CELL INHIBITION: A NEW APPROACH FOR THE TREATMENT OF MYOCARDIAL INFARCTION

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Background. Heart attacks, or Myocardial Infarction (MI) are a leading cause of cardiovascular deaths. While current treatments focus on restoring blood flow to the heart, the subsequent inflammation caused from reperfusion can lead to further damage, impairing heart function and increasing the risk of heart failure. To date, no therapies specifically target this inflammation, highlighting a crucial clinical challenge.

Methods/Results. We induced cardiac ischemia-reperfusion injury (IRI) in mice by temporarily blocking and restoring blood flow to the heart. 7 days post-IRI, we found increased inflammation within ischemic hearts compared to naïve hearts. We observed increased CD4+ effector memory T cell population locally within the heart (4.0 ´ 103, $p<0.05$) and systemically in the heart-draining mediastinal lymph nodes and spleens (3.5 ´ 103 and 7.0 ´ 105 respectively, $p<0.05$, $p<0.01$). This was also observed in activated CD4+ T cells and pro-inflammatory T cells. To investigate the impact of T cells on heart recovery, we administered abatacept, which blocks the signals required for T cell activation. Echocardiography was performed weekly for 4 weeks, and heart function parameters were measured. Abatacept-treated mice showed significant improvements in heart function such as ejection fraction (35% vs 45%, $p<0.01$, $p<0.0001$), fractional shortening, stroke volume and cardiac output. Additional strain analysis also revealed enhanced heart movement and synchronization following treatment.

Conclusion. Our findings indicate that by inhibiting T cell activation, heart function was remarkably improved following IRI. This offers new insights into the complex mechanisms underpinning MI-induced inflammation and highlights the critical importance of targeting inflammation for future therapeutic interventions.

abs #069

Harnessing Platelet-Targeted Long Circulating Thrombolysis for the Prevention of Clots and Ischemia

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Background. Existing antithrombotic drugs have limitations due to bleeding risks and short efficacy. To overcome these challenges, we developed a novel treatment called targeted long-circulating protein (Targ-LC-TAP).

Methods/Results. Targ-LC-TAP combines a platelet-targeting antibody, a human serum albumin for extended circulation, and a tick anticoagulation peptide (TAP) to inhibit clotting. A non-binding control of Targ-LC-TAP (Non-Targ-LC-TAP) was used as the control. Our experiments confirmed Targ-LC-TAP's ability to bind specifically to activated platelets and effectively prevented platelet aggregation and thrombosis in vitro. In an acute thrombosis murine model, Targ-LC-TAP significantly improved blood flow in the carotid artery compared to the Non-Targ-LC-TAP (2340 vs 1104, AUC, P=0.0029), providing up to 16 hours of thrombo-prophylactic benefits. In another murine model, we recapitulated the clinical scenario of patients with MI undergoing recanalization and demonstrated that the administration of Targ-LC-TAP prior to injury preserved cardiac function, as evidenced by increased ejection fraction compared to the Non-Targ-LC-TAP group (53 vs 31, %, P<0.0001). Strain analysis showed a reduction in the deformation of the myocardium and the histology of the left ventricle further supported a reduced infarct size for those treated with Targ-LC-TAP, as compared to PBS control (23 vs 6, P=0.0001). Importantly, as opposed to the currently used medication Clexane, Targ-LC-TAP did not prolong bleeding due to the targeted approach which enables the administration of lower doses (268 vs 1800, sec, P<0.0001).

Conclusion. Overall, Targ-LC-TAP shows great promise as a safe and effective long-lasting thrombo-prophylactic drug, offering a potential strategy to broaden the therapeutic window and improve patient outcomes in the prevention of blood clots.

abs #076

Understanding the Mucin-type O-glycan Biosynthesis in Mucosal Diseases

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Background. The mucus forms the first line of the host immune system by forming a hydrogel that overlays epithelia. However, mucus disorder is found in many mucosal diseases, such as adenocarcinoma. The polymeric glycoprotein mucin determines the mucus biology, where the mucin-type O-glycans are terminated with an α-GlcNAc residue installed by the glycosyltransferase A4GNT. Our study seeks to understand the biosynthesis and regulation of α-GlcNAc-terminated glycans on mucosal proteins and how this impacts mucus biology in health and disease.

Methods/Results. The catalytic domain of human A4GNT was recombinantly produced in insect cells, and the recombinant protein was then used for enzymology studies. The substrate preference of A4GNT was revealed by in vitro enzyme activity assays using a range of acceptor sugar substrates. The Michaelis-Menten kinetics was then determined for each substrate. Our data showed that A4GNT has a high specificity towards β-linked Gal against the α-linked one. The enzyme displayed a preference towards a β-1,3-linked Gal rather than a β-1,4-linked one, while similar catalytic efficiencies were observed for the linear and branched substrates. To understand how A4GNT functions in vivo, we also performed confocal microscopy studies with A4GNT-transfected HT29 cells. Our immunochemistry data revealed that A4GNT localizes at the trans-Golgi compartment towards the end of the O-glycan biosynthesis pathway.

Conclusion. Our enzymology and imaging studies showed that α-GlcNAc biosynthesis occurs in a range of sugar substrates while the enzyme preference matches the local substrate concentration. Such a mechanism highlights the importance of subcellular compartmentation in studying enzyme biology.

abs #080

Plasma-associated metabolomic variation in Juvenile idiopathic arthritis is not exclusively driven by chronic inflammation.

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Background. Juvenile idiopathic arthritis (JIA) is an autoimmune disease of unknown cause, characterised by inflammation of the joints in children. Although generally classified into subtypes based on clinical features, diagnosing JIA is challenging as symptoms are shared across subtypes and with other inflammatory diseases. Despite limited metabolomic analysis in JIA, the potential of metabolomics to improve diagnosis has not been fully explored.

Methods/Results. Method: A total of 73 children with JIA and 18 age/sex matched controls were selected from the CLARITY (ChiLdhood Arthritis Risk factor Identification sTudY) cohort. The metabolomic profile of circulating plasma was generated using NMR (Nightingale Health, Finland) and the association with JIA, JIA subtype, and inflammatory status (Glycoprotein acetyls, GlycA) was tested using linear regression models.

Results: We characterised the metabolomic profiles in four JIA subtypes; oligoarticular, RF-positive and RF-negative polyarticular, and systemic JIA. In total 2/249 metabolomic biomarkers were significantly different in plasma from children with JIA compared to controls. No subtype-specific metabolomic variation was detected other than for 17 biomarkers in systemic JIA. 11/249 metabolomic indicators were associated with GlycA. Only one biomarker was both JIA-specific and GlycA-associated. 4/17 systemic JIA biomarkers were associated with GlycA.

Conclusion. The identification of JIA subtype-specific biomarkers in circulating plasma supports the heterogeneity of systemic and non-systemic JIA. While GlycA is elevated in JIA plasma, the low overlap between GlycA-associated and JIA-specific biomarkers, suggests that GlycA, the hallmark of chronic inflammation, is not the exclusive factor that contributes to the altered metabolic profile in children with JIA.

abs #087

"People are tired of using guidelines": A qualitative study of facilitators and barriers to spasticity guideline implementation

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Background. Questions: What are the facilitators and barriers to implementing guideline recommendations in a spasticity clinic setting? What is the influence of focal muscle spasticity guidelines on the decisions made within a spasticity clinic?

Design: A qualitative study based on a phenomenological approach using individual interviews.

Participants: Sixteen experienced multi-disciplinary clinicians providing specialised care to people in twelve different spasticity clinics.

Methods/Results. Methods: Clinicians participated in semi-structured interviews. Two independent reviewers performed line by line coding of transcripts. Reflexive thematic analysis was undertaken with themes/sub-themes inductively derived.

Results: Seven key themes emerged. First, knowledge of specific guideline recommendations varied between clinicians. Second, health services did not prioritise resources to enable clinicians to align their practice with guideline recommendations. Third, a limited evidence base for guideline recommendations affected clinicians' willingness to implement them. Fourth, peer support was highly valued but opportunities to collaborate were limited. Fifth, a large amount of intrinsic motivation and personal time was required from clinicians if they were to successfully implement guideline recommendations. Sixth, the standardisation of clinic processes was one way in which clinicians felt they could better align their clinical practice to guidelines. Lastly, guidelines overall had an influence on spasticity clinic processes.

Conclusion. Knowledge of recommendations varied but, overall, guidelines had an influence on clinic processes and staff perceptions across the state-wide services. Health service resources, limited evidence for guideline recommendations and time constraints were considered barriers to spasticity guideline implementation. Multi-disciplinary teamwork, the individual's motivation to change and inter-clinic collaboration were considered to be the facilitators.

abs #093

Australian pluripotent stem cell lines the invisible resource

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Background. Community debate in the 1990s and early 2000s and public submissions to a Federal Parliamentary inquiry called for licensing of embryo research and establishment of a national stem cell bank or registry in Australia. This country now has an embryo research licensing committee but not a registry of pluripotent lines. Australian researchers do have the choice of international registries like hPSCreg (https://hpscreg.eu/). The limited adoption of these registries by Australian researchers and the absence of a national catalogue have led to a scarcity of information on Australian hPSCs. This study explores how these historical events have shaped common Australian practises, and seeks to understand the potential value of a local registry.

Methods/Results. Semi-structured interviews with Australian stem cell researchers and manufacturers has been conducted (47 interviews in total, based on our preliminary scoping using existing networks). Prior to the interviews, I asked participants to complete a short survey with basic demographic data and information about their experience with the existing registry to help inform the interviews.

Conclusion. 1) Australian researchers are unaware of registration as best practise, and seldom utilize international registries.
2) International visibility of Australian lines relies on publication rather than registration.
3) The community is willing to explore registration but is concerned about burdensome red tape.

abs #116

Identifying features of lymphocytes in newly diagnosed T1D BANDIT participants after treatment with the JAKi baricitinib

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Background. The BANDIT trial investigates the efficacy of baricitinib in 91 new-onset T1D. Changes in T-cell activation, regulation or exhaustion have been observed in previous T1D studies, along with changes in NK cell numbers during baricitinib treatment. The aim of this study is to examine NK cell and T-cell populations to understand how inhibiting JAK1/2 with baricitinib modifies T1D-relevant immune cell markers.

Methods/Results. Blood samples were collected and processed at baseline and after 24 weeks of treatment with baricitinib. At 24 weeks, samples were collected 2h and 24h after baricitinib. The phosphorylation of STAT1 and STAT3 in response to IFNy and IL-21 respectively were measured in fresh blood by phosphoflow cytometry as a pharmacodynamic readout of drug activity. Frequencies of lymphocyte subpopulations were also analysed using spectral flow cytometry.
The frequency of CD56bright and CD56dim NK cells decreased after 24 weeks of baricitinib (Wilcoxon test, p=0.0052) (Wilcoxon test, p=<0.0001). There was a significant reduction in the pSTAT1 and pSTAT3 in CD8+ T-cells at 24 weeks of treatment when comparing baricitinib and placebo 2h after drug intake (Komolgorov-Smirnoff test, p<0001 and p=0.0218 respectively).

Conclusion. Advances in the understanding of cytokine signal transduction led to the therapeutic targeting of JAKs to treat autoimmune diseases. Our results showed a significant reduction in the pSTAT1 and pSTAT3 in CD8+ T-cells 2h after treatment. Furthermore, our data after 24 weeks of treatment with baricitinib are consistent with data in NOD mice showing a reduction in the number of NK cells and CD8+ effector memory T-cells after JAK inhibitors.

abs #123

Towards the development of non-antibiotic based strategies to clear Klebsiella pneumoniae from the gut

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Background. Antimicrobial resistance and multidrug resistant organisms (MDROs) are a significant threat to global health. With few new antibiotics in the development pipeline, novel strategies to combat MDROs are urgently needed. Gut colonisation is a critical first step towards life-threatening infections caused by many MDROs such as carbapenem-resistant Klebsiella pneumoniae (CRE-Kp). Despite this, no therapeutics that can clear MDROs from the gut exist.

Methods/Results. Modulation of the gut microbiota offers a promising strategy to remove MDROs from the gut, but significant knowledge gaps remain. This project will address these knowledge gaps by i) understanding genetic mediators in CRE-Kp using a colonisation model, ii) identify and characterise inhibitory bacterial species of the human gut microbiome against CRE-Kp using an integrative multi-omics approach and iii) understand mechanisms of bacterial species inhibition and potential therapeutic activity in vivo and in vitro.
We have developed miniaturised high throughput assays (co-culture, spot overlay and supernatant) to screen 1034 human gut commensals strains. From this, 49 commensal strains were found to inhibit in vitro growth of CRE-Kp and the mechanism of inhibition was further analysed. This project also uses a systems level approach to understand microbiome dynamics and community structure that correlate with CRE-Kp colonisation to guide the development of multi-species probiotics that act antagonistically to inhibit gut colonisation of CRE-Kp.

Conclusion. This will lay the foundation for the future development of microbiome-based diagnostics and precision biotherapeutics that will be useful new weapons in our ongoing fight against MDRO infections.

abs #124

Elucidating the Role of Renal Microcirculatory Abnormalities in Non-Anaesthetised Sheep with Heart Failure

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Background. Approximately 50% of patients with heart failure develop chronic kidney disease (CKD), which significantly increases morbidity and mortality. There are currently no effective biomarkers or therapies for heart failure-induced CKD due to a limited understanding of its early pathophysiological mechanisms. We aimed to determine the contribution of renal macro- and micro-circulatory perturbations in a non-anaesthetised sheep model of myocardial infarction-induced heart failure.

Methods/Results. Merino ewes underwent either a myocardial infarction via ligation of the 2nd diagonal branch of the left anterior descending coronary artery (N=3) or sham surgery (N=4). In a second surgical procedure, a flow probe was implanted on the left renal artery to measure renal blood flow and fibre-optic probes were inserted in the renal cortex and medulla to assess regional kidney tissue perfusion and oxygenation in non-anaesthetised heart failure and sham-operated sheep. Development of mild-to-moderate heart failure in sheep was characterized by a 20% reduction in left ventricular ejection fraction and a 40% increase in heart rate. Renal blood flow and renal cortical tissue perfusion and oxygenation were not significantly different between heart failure and sham-operated sheep. However, there were selective reductions in renal medullary tissue perfusion (479 ± 39 vs. 1327 ± 373 blood perfusion units) and tissue oxygenation (27.7 ± 7.0 vs. 40.2 ± 6.3 mmHg) in heart failure compared with sham-operated sheep.

Conclusion. Renal medullary hypoperfusion and hypoxia can occur independently of renal macro-circulatory changes in heart failure, which could drive the progressive renal functional deficits culminating in CKD.

abs #125

Emotional Paradox: A qualitative interview study into carer's experience of fostering

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Background. Foster care is western nations preferred option for children who cannot safely reside with their parents or within their family or social networks. Foster families are perceived as best able to meet children's nurturing and emotional needs and prepare them for successful adulthood. The foster care system however faces a chronic shortage of foster carers and lack of stable placements. This research is part of a larger study around carer retention and placement sustainability. It explores how carers experience their roles and the social and individual factors that motivate them to care.

Methods/Results. Fourteen carers were interviewed on their expectations and experiences of foster caring. Seven professionals were also interviewed around their observations of carers experiences.

Conclusion. This study reveals carers are motivated to care for significant personal reasons however experience contradictory expectations from the state and many pay an extreme personal cost for caring. These findings challenges the traditional way of regarding carers and how they are supported in their role.

abs #136

Analysing non-proteinaceous ubiquitination by mass spectrometry

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Background. Ubiquitin is an essential eukaryotic protein that can be covalently attached to other molecules in complex combinatory ways. It is involved in the regulation of almost every cellular process and therefore plays a role in a large number of human diseases, including various cancers, infections or neurodegenerative and autoimmune diseases. Recent studies have described the ubiquitination of lipopolysaccharides (LPS), sugars and lipids, expanding the role of ubiquitin modifications beyond proteins. However, methodologies to investigate non-protein ubiquitination have so far been hampered by a lack of suitable tools and reagents.

Methods/Results. In this study, we explore non-proteinaceous ubiquitination through the development and use of a new, unbiased, mass spectrometry-based workflow. A key component of this method is the use of the viral and bacterial proteases Lbpro and Jopro, which cleave ubiquitin at its C-terminus and leave characteristic GlyGly marks on ubiquitinated substrates. We then enrich for GlyGly-modified small molecules, including non-proteins, via molecular weight filtering and identify them by ammonium acetate/acetonitrile based liquid chromatography (LC) connected to a quadrupole-orbitrap mass spectrometer (MS). GlyGly-characteristic mass-shifts and MS2 fragmentation are used to infer the identity of a molecule. At this stage, our method can robustly detect in-vitro catalysed ubiquitinated sugars that were spiked into various cell lysates.

Conclusion. We established and optimized an LC-MS-based protocol that can detect ubiquitinated non-proteins. The method can effectively identify in vitro catalysed ubiquitinated sugars and presumably a wide range of other ubiquitinated non-proteins. This tool gives the opportunity to unravel novel biological mechanisms and pathways in unprecedented ways.

abs #159

Natural Language Processing Diagnosed Behavioural Disturbance Phenotypes in the ICU: Characteristics, Prevalence, Trajectory, Treatment, and Outcomes

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Background. Natural Language Processing (NLP) may help evaluate the characteristics, prevalence, trajectory, treatment, and outcomes of behavioural disturbance phenotypes in critically ill patients.

Methods/Results. We obtained electronic clinical notes, demographic information, outcomes, and treatment data from three medical-surgical ICUs. Using NLP, we screened for behavioural disturbance phenotypes based on words suggestive of an agitated state, a non-agitated state, or a combination of both.

We studied 2,931 patients. Of these, 225 (7.7%) were NLP-Dx-BD positive for the agitated phenotype, 544 (18.6%) for the non-agitated phenotype and 667 (22.7%) for the combined phenotype. Patients with these phenotypes carried multiple clinical baseline differences. On time-dependent multivariable analysis to compensate for immortal time bias and after adjustment for key outcome predictors, agitated phenotype patients were more likely to receive antipsychotic medications (odds ratio [OR] 1.84, 1.35-2.51, p<0.001) compared to non-agitated phenotype patients but not compared to combined phenotype patients (OR 1.27, 0.86-1.89, p= 0.229). Moreover, agitated phenotype patients were more likely to die than other phenotypes patients (OR 1.57, 1.10-2.25, p=0.012 vs non-agitated phenotype; OR 4.61, 2.14-9.90, p<0.001 vs combined phenotype). This association was strongest in patients receiving mechanical ventilation when compared with the combined phenotype (OR 7.03, 2.07-23.79, p=0.002). A similar increased risk was also seen for patients with the non-agitated phenotype compared with the combined phenotype (OR 6.10, 1.80-20.64, p=0.004).

Conclusion. NLP-Dx-BD screening enabled identification of three behavioural disturbance phenotypes with different characteristics, prevalence, trajectory, treatment, and outcome. Such phenotype identification appears relevant to prognostication and trial design.

abs #174

Quantitative susceptibility mapping of infarct tissue response in post-ischemic stroke patients

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Background. Quantitative Susceptibility Mapping (QSM) is a valuable imaging tool for quantifying subtle variations in magnetic susceptibility. QSM has been used to quantify brain iron , a marker of metabolic activity or tissue damage in stroke patients (Langkammer et al 2012, Neuroimage)(DeGregorio-Rocasolano et al 2019, Front Neurosci). Preliminary animal and human stroke studies using QSM have shown brain iron differences between infarct and unaffected tissue (Vaas et al 2018, Translational Stroke Resesarch) (Probst et al 2021, Scientific Reports) (Dimov et al 2021, Magnetic Resonance in Medicine). Our aim is to use QSM to assess longitudinal tissue changes in brain MRI images of stroke patients post-treatment.

Methods/Results. 23 ischemic stroke patients with successful reperfusion treatment underwent MRI scans at 1-3 days (tp 1), 3 months (tp 2) and 12 months (tp 3) post-reperfusion to obtain diffusion-weighted (DWI), fluid- attenuated inversion recovery (FLAIR), and multi-echo gradient-recalled echo (GRE). GRE images which were used to generate QSM images. ROIs delineating the infarct and unaffected mirror regions were drawn from which QSM values were extracted and compared statistically. Statistical significance was established at $p < 0.05$.

QSM values in the infarct ROIs increased from tp 1 to tp 3, significantly differing between tp 1 and tp 2 ($p = 0.007$), as well as tp 1 and tp 3 ($p = 0.0005$) but not between tp 2 and tp 3 ($p = 0.236$). Unaffected ROIs showed consistent QSM values over time.

Conclusion. QSM can adequately track post-stroke tissue changes. The increasing QSM values, signifying increasing iron deposition could imply either further neurodegeneration or neural repair and regeneration. Further research with other tissue change measures is necessary to understand the significance of iron deposition.

Cancer

abs #003

Investigating the expression and metabolite antigen presentation of MR1 in cancer

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Background. Unconventional T cells, including mucosal-associated invariant T (MAIT) cells, bridge innate and adaptive immunity. Antigen (Ag) presentation by major histocompatibility complex class I-related protein 1 (MR1) results in MAIT cells effector functions including cytokine release. Some studies suggest MAIT cells contribute to anti-cancer immunity, while others indicate they promote tumour progression. Also, tumours can present Ags on MR1 that are recognised by non-MAIT MR1-restricted T cells. Therefore, it is important to understand MR1 antigen presentation in the tumour microenvironment.

Methods/Results. A novel MR1 reporter mouse model was generated in our lab to overcome the difficulty of detecting the low-level expression of MR1. This model uses the tdTomato fluorescent protein to report MR1 transcription (MR1TOM), enabling the assessment of MR1 expression in cancer models. To investigate MR1 expression in the context of lung metastases, B16.F10 melanoma cells were injected intravenously into MR1TOM, or WT C57BL/6 mice and tissues harvested at day 12. Analysis of tdTomato expression (MR1Tom mice) and MR1 protein (WT mice) was determined by flow cytometry. The expression of MR1TOM was significantly reduced in alveolar macrophages (AMs) during lung metastases, while other cell types show minimal changes. The MR1 protein expression in WT mice, assessed through surface expression after ligand upregulation, was more pronounced, where MR1 protein became undetectable in AMs after tumour development.

Conclusion. Our findings demonstrate a significant downregulation of MR1 expression in AMs during lung metastases. As AMs are the cell type expressing the highest level of MR1 in the lung, downregulation of MR1 after lung metastases might disrupt the interaction between MR1 and MAIT cells in the cancer context. Further investigations into the underlying mechanisms and functional implications of MR1 downregulation in cancer may provide valuable insights into tumor-immune interactions and potential therapeutic strategies.

abs #031

Evaluating the role of AP-1 transcription factors in CD8+ T cell differentiation and fate

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Background. Enhancing adoptive cell therapies to boost their anti-tumour effectiveness is a major focus of the cancer immunotherapy field. One approach gaining interest is overexpressing the transcription factor, AP-1, in CAR T cells to overcome regulatory processes like exhaustion. AP-1 is a family of dimeric TFs, primarily composed of Jun and Fos proteins, which is assumed to play a crucial role in T cell differentiation through both promoting cytokine production and suppressing regulatory processes. However, there is surprisingly little functional evidence to support these assumptions. In fact, given the large number of AP-1 family members, no study has fully knocked out AP-1 to assess its contribution to CD8+ T cell differentiation in vivo.

Methods/Results. We recently developed a novel CRISPR-Cas9 gene editing method enabling rapid generation of knock-out (KO) T cells. Using this technique, we optimised an approach to fully ablate all Jun proteins (i.e. cJun, JunB and JunD) in naïve CD8+ T cells, thereby eliminating all potential AP-1 complexes. During acute viral infection, AP-1 KO CD8+ T cells display a survival defect compared to control cells, with the surviving cells also having decreased expression of immune memory markers and increased expression of exhaustion markers. Unlike exhausted cells, though, KO cells retained cytokine effector function, suggesting that AP-1 may be fully dispensable for T cell cytokine production. KO cells also failed to acquire features of peripheral tolerance, a regulatory state that AP-1 was proposed to suppress. Interestingly, KO of JunB alone was sufficient to recapitulate the AP-1 KO cell phenotype, with KO of c-Jun and/or JunD having little impact on T cell differentiation.

Conclusion. Thus, contrary to current dogma, AP-1 represses aspects of the exhausted fate but does not impact peripheral tolerance or acquisition of T cell effector functions. Moreover, only JunB-containing AP-1 complexes appear functionally important in normal effector T cell differentiation.

abs #035

The UNC5B Dependence Receptor regulates colorectal cancer stem cell self-renewal and apoptosis

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Background. Dependence receptors are characterized by the activation of two distinct signalling pathways depending on the presence or absence of their ligand. Ligand binding triggers signalling pathways that promote cell survival and proliferation, while the absence of ligand actively triggers apoptosis. This study aimed to investigate the impact of the Netrin-1 dependence receptor UNC5B on cell plasticity and apoptosis of metastatic colorectal cancer cells, using a patient-derived tumour organoid model.

Methods/Results. Using Extreme Limiting Dilution Assays, we found that Netrin-1 significantly increased self-renewal in organoid lines derived from metastatic patient tumors. Conversely, inhibition of Netrin-1 using a clinically tested neutralizing antibody, NP137, drastically decreased self-renewal. We further demonstrated that this NP137-induced reduction was Unc5B-dependent, suggesting that pro-apoptotic signalling downstream from UNC5B modulates self-renewal, a key characteristic of stem cells. These results led us to hypothesize that Netrin-1 participates in the maintenance of the colorectal cancer stem cell pool by inhibiting the apoptotic cascade triggered by UNC5B. Corroborating this hypothesis, we found that NP137 treatment induced the degradation of the anti-apoptotic protein MCL1, and that treatment with an caspase inhibitor reversed the effect of NP137 on self-renewal. We are now further characterising the effect of NP137 using single-cell sequencing and evaluating the therapeutic potential of the NP137 antibody to prevent tumor recurrence in vivo.

Conclusion. This project identify the Netrin-1/UNC5B signalling axis as an important regulator of self-renewal in colorectal cancer stem cells, highlighting the potential of targeting this pathway in metastatic colorectal cancer.

abs #045

The Landscape Of On-Target, Off-Target, And Collateral Activity Of Various CRISPR-Cas13 Enzymes In Human Cells

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Background. Precise editing of cellular transcriptomes with sequence-specific RNA-targeting tools is crucial for comprehending biological processes and has the potential to transform therapeutics for various genetic disorders, including cancer.
A novel class of prokaryotic RNAi called CRISPR-Cas13 has been recently discovered. These are programmable RNA-guided RNA-targeting enzymes that exclusively degrade target single-stranded RNAs. The long spacer sequence (22-30nt) of Cas13 offers rigorous target recognition compared to classical eukaryotic RNAi and Cas9 tools. Despite stringent target recognition, some Cas13 orthologues were reported to exhibit collateral nuclease activity that mediates indiscriminate degradation of nearby host RNA molecules. This process was described in bacteria and further harnessed in-vitro. However, human cells have a more complex cellular organization and compartmentalized architecture, which may affect on-target and collateral activities of Cas13. Currently, the extent of different Cas13 orthologues' collateral activities in human cells remains unclear in literature.

Methods/Results. Here, we aimed to investigate the targeting efficiency and specificity of different Cas13 orthologues in human cells. We used quantitative fluorescence, western blotting, and mass spectrometry to evaluate on-target and collateral nuclease activities of Cas13 enzymes.
All Cas13 orthologues we tested demonstrated high on-target silencing. Notably, for RfxCas13d, we observed prominent collateral activities directed only against over-expressed exogenous transcripts but not endogenous transcripts. In contrast, PspCas13b and LwaCas13a orthologues exhibited potent degradation of target RNA with no detectable collateral activities.

Conclusion. Overall, this study revealed that Cas13 enzymes present high targeting fidelity in human cells, highlighting their potential to precisely target oncogenic driver transcripts in cancer therapeutics and beyond.

abs #052

Investigating the Metabolic Regulation of Lysosomal Biogenesis

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Background. Cancer cells depend on an adequate nutrient supply to fuel the biosynthetic processes driving their uncontrolled growth. However, nutrient availability in the tumour microenvironment is frequently compromised due to poor vascularisation and competition for resources in the tumour niche. Therefore, cancer cells often need to employ alternative approaches to acquire nutrients. Central to many of these nutrient acquisition pathways is the lysosome, an organelle that breaks down macromolecules into their constituent parts. Malignant transformations are associated with various changes to the lysosome, including their biogenesis. Lysosomal biogenesis is controlled by TFEB/TFE3, transcription factors that regulate key proteins in lysosomal function. Emerging evidence suggests that cancers may exploit TFEB/TFE3 to fuel their growth and promote survival. However, the molecular mechanisms that underpin this exploitation are poorly understood.

Methods/Results. Using physiologically relevant cell culture models, we have utilised the deprivation of amino acids to promote lysosomal biogenesis driven by TFEB/TFE3. We have performed a targeted metabolic CRISPR screen to identify genes that contribute to the regulation of lysosomal biogenesis under these stress conditions. We have identified and validated two genes from this screen, MSRA and FN3K, which, when deleted, exacerbate lysosomal biogenesis. There is evidence for these genes and their function in the context of cancer, however, there is no established link between their role and lysosomal biogenesis.

Conclusion. We are currently exploring the mechanisms that drive and promote lysosomal biogenesis following the targeted deletion of these genes in our model. Our study hopes to gain an understanding of the broader regulation of lysosomal biogenesis as well as their contribution to tumour progression through currently unexplored pathways.

abs #062

Cardioprotective effects of a novel Drp1 inhibitor in anthracycline-induced cardiotoxicity

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Background. Anthracyclines (e.g., doxorubicin) are an effective chemotherapeutic option to treat cancers but their usage may cause heart failure. Unfortunately, there is no effective treatment for doxorubicin-induced cardiotoxicity. Dynamin-related-protein-1 (Drp1) is a key protein that maintains mitochondrial health and function. Pre-clinical studies reported that increased Drp1 levels favour cancer cell survival and mediate doxorubicin-induced cardiotoxicity. It is hypothesised that Drp1 inhibition will enhance doxorubicin-based cancer treatment while reducing doxorubicin-induced cardiotoxicity. Recently, our lab discovered a specific inhibitor of human Drp1 protein called OB37.

Methods/Results. The anti-cancer effects of OB37 were assessed using 2D and 3D cultures of human osteosarcoma (MG63) and ovarian cancer (OVCAR3) cells. In 2D osteosarcoma, OB37 (5-50μM for 5 days) significantly increased cell death compared to control (0.1% DMSO), and OB37 (50μM) also synergised with doxorubicin in promoting further cell death ($n=6$-9, $P<0.05$). In ovarian cancer cells, OB37 significantly increased cell death when cultured under 3D conditions ($n=6$, $P<0.05$), but had no effect under 2D conditions ($n=6$, $P>0.05$). The cytoprotective effects of OB37 against doxorubicin-induced cardiotoxicity were assessed using 2D and 3D cultures of cardiomyocytes derived from human-induced pluripotent stem cells. In 2D cardiomyocytes, treatment with OB37 (5-50μM for 2 days) did not affect cell viability or protect cells from doxorubicin-induced cardiotoxicity ($n=6$-9, $P>0.05$). Excitingly, OB37 (5-50μM for 2 days) significantly protected 3D cardiomyocyte spheroids against doxorubicin-induced cardiotoxicity by reducing the release of lactate dehydrogenase, a surrogate marker of cell injury ($n=3$, $P<0.05$).

Conclusion. OB37 showed anti-cancer effects in osteosarcoma and ovarian cancer cells and cytoprotective effects against doxorubicin-induced cardiotoxicity.

abs #065

Using spatial transcriptomics to locate T cell subsets in human cancer tissue

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Background. Noncirculating tissue-resident memory T cells (TRM) can be present at the border of tumours, maintaining a tumour-immune equilibrium to prevent tumour outgrowth. Importantly, peritumoral TRM retain higher functionality compared to tumour-infiltrating T cells that can become exhausted and dysfunctional (TEX). Independently, TRM transcriptional signatures have been associated with improved long-term survival across many tumour types. Therefore, incorporating spatial information into the current analysis pipeline will allow us to interrogate the development of tumour-associated TEX and TRM respectively within the actual tissue structure.

Methods/Results. T cells are one of the smallest cell types. As such, most spot-based sequencing techniques were unable to identify T cell subsets accurately. Here, we leveraged the in-situ sequencing technology, Xenium In Situ which sequences cells without dissociating them thus accurately preserving cellular location. This provides a much greater ability to differentiate T cell subsets. Using signatures generated from our laboratory's single-cell RNA sequencing of human breast cancer T cells, we explored techniques to locate these T cell subsets within publicly available breast cancer Xenium tissues. We observed that TRM generally reside outside the tumour region where there is more heterogeneity of different cell types while dysfunctional TEX are found more frequently within cancerous regions.

Conclusion. This research will further explain the molecular mechanisms related to the development of TRM and TEX and their interactions within the tumour microenvironment, which is of high clinical interest. Specifically, this will be one of the first studies to explore subsets of T cells using in-situ sequencing technology spatially.

abs #075

Developing Next Generation Armoured CAR T cells via CRISPR Knock-in

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Background. Chimeric antigen receptor T (CAR-T) cell therapy has shown tremendous success in haematological malignancies but limited efficacy in solid tumours, owing to immunosuppressive tumour microenvironment, tumour heterogeneity and inefficient tumour trafficking. One promising attempt to overcoming these includes “armouring” CAR-T cells with a therapeutic transgene. We previously demonstrated that CAR-T cells engineered to secrete dendritic cell growth factor Flt3L could effectively engage the host immunity, which is critical in overcoming antigen-negative relapse[1]. However, artificial promoter systems have demonstrated insufficiencies in achieving site-specific transgene expression, which had caused systemic toxicities and trail termination[2]. The advent of CRISPR/Cas9 gene-editing has enabled the precise engineering of CAR-T cells for safety and efficacy enhancements. We showed that CRISPR knock-out (KO) of immunosuppressive gene A2AR enhanced CAR-T cell function[3]. Now, we aim to exploit CRISPR knock-in (KI) of transgenes into tumour-specific gene loci for tighter regulation of transgene expression.

Methods/Results. We performed genome-wide RNA-Sequencing on CAR-T cells and identified 27 genes with tumour-specific expression as potential KI sites. As target gene expression is disrupted during KI, we first assessed the impact of each gene KO on CAR-T cell function/phenotype. Subsequently, 7 genes that did not adversely impact function/phenotype following KO had GFP knocked in. RGS16 and NR4A2 emerged as novel promoters that upon KI elicit enhanced transgene expression in tumours and lower transgene expression at non-tumour sites relative to the prototypic PD-1 promoter.

Conclusion. Our findings enabled the generation of armoured CAR-T cells with higher cytokine-secreting capacity specifically in tumours and thus improved safety and efficacy profiles.

abs #077

Effect of non-anticoagulant heparin on oral squamous cell carcinoma

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Background. Oral squamous cell carcinoma (OSCC) is a global concern with alarming prevalence and poor survival rates. Anticoagulants like heparin show potential for improving cancer survival but pose challenges due to bleeding risk. Non-anticoagulant heparin (NAH), a novel derivative, offers a promising solution.

Methods/Results. We used the MTS proliferation assay (0/24/48/72hrs), scratch assay (MuviCyte™ Live-Cell Assay Imaging System, 0-18hrs), and invasion Matrigel™ (24hrs) to assess the in vitro effects of NAH (10-20U/mL), and heparin (10-20U/mL) on two human OSCC (H400/H357) and one normal keratinocytes (OKF6) cell lines and their ability to interfere with the commonly used chemotherapeutics 5-FU (1-5 µg/mL) and cisplatin (1-5 µg/mL). Remarkably, non-anticoagulant heparin (NAH) significantly reduced the proliferation of H400 and H357 cells at 48 and 72 hours, respectively, to a comparable extent as heparin. Notably, neither drug exhibited cytotoxic effects on normal keratinocytes at the tested time points. Furthermore, both heparin and NAH significantly inhibited cell migration in H400 and H357 cells within 12 hours. Additionally, both drugs affected the invasion phenotypes of the tested cancer cell lines. Importantly, the addition of these drugs to commonly used chemotherapeutic agents for OSCC did not compromise their efficacy against the tested cell lines. Surprisingly, treatment of OKF6 cells with NAH significantly reduced the cytotoxic effects of chemotherapy.

Conclusion. In conclusion, non-anticoagulant heparin (NAH) demonstrates promising potential as an alternative to heparin for improving cancer survival in oral squamous cell carcinoma (OSCC). NAH significantly reduced proliferation and inhibited migration in OSCC cells, without compromising the efficacy of commonly used chemotherapeutic agents 5-FU and cisplatin.

abs #098

Understanding heterogeneity of PSMA in metastatic prostate cancer

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Background. Prostate-specific membrane antigen (PSMA) is an essential target for treating metastatic castration-resistant prostate cancer (mCRPC), with radioligands aiming to address its overexpression on tumours. However, 30% patients have negative or low PSMA and therefore cannot receive this treatment. Combination treatments aiming to reactivate expression of PSMA offer renewed hope. Unfortunately, regulatory mechanisms of PSMA remain unclear.

Methods/Results. We used single-nuclei RNA sequencing on 9 mCRPC patients with 32 samples from various sites. PURPLE called copy number variants (CNVs) from whole genome sequencing. We re-defined intra-tumour phenotypes and conducted differential analysis and function enrichment to reveal relating pathways. Firstly, we found FOLH1-low phenotype persisted without obvious CN deletion on FOLH1 (PSMA encoding gene), thus CNVs were not the dominate influencer. Despite a general positive correlation between AR and FOLH1, anti-correlation presented in some samples, which encouraging redistribution of cell populations using AR/ FOLH1 expression. Intra-tumour heterogeneity was highlighted by presence of AR-high/FOLH1-low and AR-low/FOLH1-high phenotypes within tumours. Functional enrichment on differential expressed genes between FOLH1 gain and loss uncover distinct pathways in varying AR context. Therefore, FOLH1 loss with high AR was linked hypoxia metabolism while low AR cases had more interaction with immune pathways.

Conclusion. In conclusion, cells losing PSMA expression may experience a metabolic switch and communicate differently with surrounding immune microenvironment, opening the possibility of combining metabolic regulators and immunotherapy with PSMA-targeted radioligands to treat patients with reduced PSMA.

abs #115

Elucidating the prognostic and functional roles of NK cell subsets in bladder cancer

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Background. Anti-tumour immune responses from NK cell were reported in bladder cancer (BLCA), however, the distinct anti-tumor functionalities of CD56bright and CD56dim NK subsets against BLCA remain largely unexplored. In this study, we elucidated the prognostic implications of these NK subsets in BLCA through a computational approach.

Methods/Results. Unique molecular signatures of CD56bright and CD56dim NK cells dissected the relative abundances of 3 stromal and 20 immune cells in the patient tumour transcriptomes from TCGA-BLCA dataset. CD56bright NK cells was predicted as the abundant NK subsets, which is also associated with improved patient prognosis. A similar favorable survival trend was projected for the mature-myeloid-dendritic (mDC) cells and CD8+-effector-memory-T cells. Presence of a potential crosstalk between NK-mDC-CD8+T cells was recorded. Expression of transcripts encoding the activating NK cell receptors such as NKG2D, NKp44, CD2, and CD160 showed positive survival trends in combination with CD56bright NK cell infiltration. Transcription factors such as HOBIT, IRF3, and STAT2 were also correlated with CD56bright NK cell abundance. Additionally, HOBIT-dependent tissue-residency program was potentially correlated with NK/T cell signatures. Expression of different immune checkpoint receptors was found to be associated more with T cell signatures than NK cells. Finally, another indipendent single cell RNAseq dataset recapitulated the findings from the bulk transcriptomes.

Conclusion. Overall, this study highlights the functional significance of CD56bright NK in BLCA patient prognosis that can potentially facilitate a better understaing of the NK cell's anti-tumour responses, which can ultimately lead to the development of promising NK cell therapies against BLCA.

abs #117

Enhancing current immunotherapies by improving T cell infiltration into solid tumours

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Background. Immune checkpoint blockade (ICB) has revolutionised the treatment of numerous cancer types, particularly those with a high mutational burden such as melanoma and non-small cell lung carcinoma. ICB targets immune-inhibitory molecules on the surface of T cells, unleashing their anti-tumour potential. Despite ICB's success, a high frequency of patients fail to respond to ICB. A key limiting factor to ICB responses is the number of T cells that can infiltrate the tumour microenvironment. T cell infiltration in the context of ICB has been shown to be dependent on attractant molecules CXCL9 and CXCL10. CXCL9 and CXCL10 are chemokines expressed by macrophages within the tumour microenvironment that attract T cells to the tumoral site. A pan-cancer meta-analysis has identified CXCL9 expression as one of the strongest predictors for a positive response to ICB. In addition, the expression of CXCL9 and CXCL10 is predictive of improved T cell infiltration and patient survival in melanoma and urothelial carcinoma cohorts that have received ICB.

Methods/Results. We have identified regulators of chemokine production by whole genome CRISPR/Cas9 screening using a By targeting chemokine regulators in combination with ICB, we hope to improve the efficacy of ICB and clinical outcomes for patients.novel reporter macrophage cell line that was generated using CRISPR/Cas9 homology directed repair. Subsequently, we have depleted these regulators in myeloid cells in vivo and observed improved intratumoral CXCL9 expression and enhanced T cell infiltration.

Conclusion. Targeting regulators of CXCL9 and CXCL10 expression improves T cell infiltration. By targeting chemokine regulators in combination with ICB, we hope to improve the efficacy of ICB and clinical outcomes for patients.

abs #129

Multi-site Retrospective Cohort Study of Central Venous Access Device (CVAD) Removal in Patients with Haematology Malignancies at four Victorian Comprehensive Cancer Centre Clinical Partner Organisations

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Background. Central Venous Access Devices (CVAD) are critical to the efficient and effective delivery of some or all aspects of cancer care for patients with haematological malignancies. However, cancer patients have some of the highest CVAD failure rates among hospitalised patients at approximately 19 to 41% resulting in increased morbidity and mortality, negative patient experiences and increased health care expenditure. This PhD set out to describe, for the first time in Australia, proportions of premature CVAD removal and removal when device no longer required, among adult haematological cancer patients in four Victorian Comprehensive Cancer Centre (VCCC) Alliance partner hospitals in a one-year period.

Methods/Results. Following completion of a scoping review, a multi-site, retrospective cohort study was undertaken to collect hospital administrative and medical record data of patients who underwent a CVAD insertion between 1 September 2020 to 31 August 2021. Data included patient demographic, disease, and treatment characteristics; insertion and device information; adverse events and removal reasons; and CVAD dwell time. An analysis of hospital administrative and medical records data is underway and will include descriptive statistics and regression modelling to identify modifiable and non-modifiable predictors of complications / premature removal and time to complication events. This paper will present preliminary analysis of data on 1116 prematurely removed CVADs across 676 patients and reasons for removal.

Conclusion. To date the study has generated data that will help standardise evidence-informed CVAD management from insertion to removal across leading heamatology centres in Victoria.

abs #140

Inhibiting PRMT5: A Novel Target for the Treatment of Melanoma

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Background. Clinical outcomes of BRAF-mutant melanoma patients have significantly improved with combination BRAF and MEK inhibitors (BRAFi/MEKi), which target components of the MAPK/ERK signalling pathway. However, the success of these targeted therapies is hampered by drug resistance; mainly contributed by overactivation of the MAPK/ERK pathway. Recently published data from our lab have demonstrated that co-inhibiting CDK4/6 and PRMT5, both downstream of the MAPK/ERK pathway, was an effective therapeutic strategy in BRAF-mutant melanoma. Hence, we hypothesised that BRAFi-resistant melanoma cells would also respond to the dual inhibition of CDK4/6 and PRMT5, considering CDK4/6 and PRMT5 lie downstream of activating events that lead to BRAFi/MEKi-resistance.

Methods/Results. Through dose-response assays, we found that melanoma cells that have increased resistance to BRAFi remain sensitive to CDK4/6 inhibitors (CDK4/6i) and PRMT5 inhibitors (PRMT5i). More importantly, co-inhibiting CDK4/6 and PRMT5 suppressed the proliferation of resistant cells in vitro and in vivo. Mechanistically, our whole genome CRISPR/CAS9 knockout screen and RNA sequencing data identified the p53 pathway as important for a robust response to PRMT5i. Correspondingly, rMATs splicing analysis identified increased exon 6 skipping in MDM4 following PRMT5i, which results in loss of full length MDM4 and thus, p53 reactivation since MDM4 is a negative regulator of p53.

Conclusion. Overall, evidence from our studies support the functional cooperativity of CDK4/6i and PRMT5i as a combination therapy, which exert their effects in part through p53 mechanisms. Thus, co-inhibiting CDK4/6 and PRMT5 serve as a potential treatment strategy for BRAF-mutant melanoma patients who are resistant to BRAFi/MEKi.

Cancer

abs #142

Novel insights into the substrate-specific DNA branch migration activity of the FANCM translocase

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Background. Our DNA is constantly damaged by external sources and products of metabolism. As DNA damage is a serious threat to cellular life, cells have evolved the ability to repair DNA damage. Insufficient DNA repair and the accumulation of mutations drives aging and diseases such as cancer. One protein that acts to suppress the development of cancer and is integral to the repair of DNA damage, and specifically DNA damage which stalls DNA replication, is FANCM. FANCM is a DNA translocase which specifically binds and remodels branched DNA structures such as a stalled replication fork with high affinity and recruits downstream DNA repair factors.

Methods/Results. Using recombinant FANCM protein and in vitro biochemical assays, we show that the N-terminal translocase domain of FANCM is responsible for the substrate recognition, and what is known as the 'branch migration activity', that allows FANCM to remodel its substrates. We also identify a domain within the translocase, the 'insert' domain, as the substrate-specificity domain which is responsible for FANCMs high affinity to branched substrates. We show that loss of the insert domain disrupts the ability of FANCM to functionally engage its substrate, and results in loss of branch migration activity. Using mutational analyses, we have also identified three residues within the insert, K331, Y332, and H369, as key residues for the specific interactions between FANCM and branched DNA.

Conclusion. Our results provide novel insight into the mechanism behind the structure-specific enzymatic activity of FANCM that underpins its DNA repair, and thus cancer-suppressive, functions.

abs #145

Sequencing CREB-regulated immunosuppression in the GBM tumour microenvironment

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Background. Glioblastoma (GBM) is the most common and aggressive adult primary brain cancer, characterised by an immunosuppressive tumour microenvironment (TME), which hinders the effectiveness of immunotherapy. Immunosuppression is largely driven by infiltrating tumour associated macrophages (TAMs), which constitute up to 30% of cells in the GBM tumour TME. Previous studies show that the expression of key immunosuppressive factors in macrophages is regulated by the kinase inducible transcription factor, cyclic AMP response element binding protein (CREB). This project investigates the role of CREB in TAM-mediated immunosuppression, in GBM.

Methods/Results. To investigate CREB activation in TAMs, multiplex immunohistochemistry was performed on 4 GBM tissue samples. To further investigate CREB-regulated immunosuppressive cytokine expression in macrophages, macrophages derived from THP-1 cells and blood monocytes were treated with GBM-conditioned media (CM) collected from a series of GBM cell lines and patient-derived GBM cells. 80% TAMs exhibit high levels of activated phospho-CREB (pCREB) expression, and most pCREBhigh TAMs were positive for M2-like biomarkers, suggesting a correlation between CREB activation and immunosuppressive TAM polarisation. Moreover, CREB activation and increased expression and secretion of immunosuppressive cytokines was observed in macrophages treated with GBM-CM, which could be blocked by a CREB inhibitor, 666-15.

Conclusion. The results demonstrate that CREB plays a key role in regulating the expression of immunosuppressive cytokines in TAMs. As CREB is crucial in GBM tumorigenesis, the results further suggest that CREB may be an effective therapeutic target to both inhibit GBM cell growth and reduce the immunosuppression in the TME. Future experiments will include transcriptomic and proteomic analysis of GBM-CM stimulated macrophages, treated with/without the CREB inhibitor and their effects on T-cell activation and cytotoxicity. This will identify pro-tumour mechanisms established by cancer-immune cell interactions in GBM tumours, which represent novel TME targets.

abs #151

The dual role of the cellular prion protein in glioma

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Background. Grade 4 gliomas (glioblastoma) are the most aggressive and frequent brain tumours, with current treatments unable to overcome poor patient prognosis and tumour recurrence. This study investigates the role of the cellular prion protein (PrPC) in glioma/glioblastoma, following observations correlating increased PrPC expression with advanced tumour grade, worse prognosis, tumour recurrence, and therapy resistance.

Methods/Results. To interrogate the role of PrPC in glioma, PrPC gene (Prnp) expression was varied in low and high-grade mouse models of glioma, targeting the PI3K/Akt pathway. In the high-grade glioma model, mice expressing wild-type (WT) Prnp developed hypercellular, invasive tumours. In contrast, tumours induced in Prnp knock-out (KO) mice were less invasive with reduced cellularity and KO mice exhibited improved survival. These findings were reflected in neural stem cells (NSCs) isolated from PrPC KO and WT high-grade glioma mice, with a significant decrease in cell proliferation observed in PrPC KO derived NSCs. Supporting the role of PrPC in cell proliferation within the high-grade glioma model and consistent with observations in tumours from glioblastoma patients, an increase in PrPC production was observed in transformed NSCs, relative to controls. These observations were markedly different in a low-grade glioma model where PrPC ablation (KO) significantly promoted NSC proliferation and stemness.

Conclusion. These results indicate both a suppressive and promoter role for PrPC in glioma, which may rely on the genetic context or grade of tumours. Understanding the role of novel factors such as PrPC in glioma may provide the basis for novel therapeutic strategies to complement limitations of current treatments.

abs #158

Sequencing BRAFi/MEKi after aPD1/aCTLA4 results in a potent immune microenvironment in Yummer 1.7 PV1 model

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Background. Targeted therapy (TT - BRAFi/MEKi) and immune-checkpoint inhibitors (ICI - aCTLA4/aPD1), two revolutionary treatments for cutaneous melanoma, are known to have their own Limitations. Hence, to increase the frequency of durable responses while circumventing the toxicity associated with the combination of ICI + TT treatments, it has been proposed that ICI and TT should be administered sequentially to patients while they are still responding to each of these treatments. However, several questions, such as the mechanism behind this synergy and the ideal sequencing approach, remain unresolved.

Methods/Results. To address these questions, we have optimized a syngeneic mouse melanoma model, YUMMER1.7-PV1. Investigating the three known sequences in this model, TT+ICI, TT-> ICI, and ICI->TT, there were no overall survival differences observed between any of the strategies, yet a longer progression-free survival was observed in TT+ICB and ICI->TT. Examination of tumor immune microenvironment (TIME) at the switch of the therapy, demonstrates that there is an immune suppressive microenvironment present in the TT first-line therapy group compared to ICI first-line therapy group. Surprisingly, this was also the case for TT+ICI group yet an outstanding number of mice were found to be disease free. Importantly, there were fewer exhausted CD8+ T-cells present at the switch of the therapy in the ICI -> TT group compared to the TT -> ICI group.

Conclusion. This evidence shows that a first-line ICI potently primes the TIME, primarily by limiting the inflow of suppressive immune cells following the switch of treatment, resulting in improved progression-free survival.

abs #160

Flt3L and CD40L-expressing CAR T cell therapy drives activation of antitumour immunity to overcome tumour heterogeneity.

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Background. Adoptive cell transfer (ACT) therapy using patient-derived T cells genetically engineered to express a chimeric antigen receptor (CAR) is highly effective in B cell malignancies and is now FDA-approved. However, tumour heterogeneity remains a major challenge in treating solid tumours due to the relapse of tumours negative for the CAR-targeted antigen. Previously we demonstrated that CAR T cells engineered to secrete dendritic cell growth factor Fms-like tyrosine kinase 3 ligand (Flt3L) promote host anti-tumour immunity by expanding intratumoural conventional type 1 dendritic cells (cDC1). A key aspect of this study was that cDC1s required a second activatory stimulus to elicit anti-tumour immunity. Here, we explored the possibility of leveraging the CD40 axis to promote cDC1 function and synergise with Flt3L-secreting CAR T cell therapy.

Methods/Results. Upregulation of co-stimulatory molecules indicating DC maturation and expansion of tumour-antigen specific T cells was observed upon anti-CD40 activation of Flt3L-expanded cDC1 in vivo. Successful engineering of CAR T cells was conducted using a novel construct to induce high CD40L expression constitutively. Improved DC maturation was observed upon CD40 engagement following adoptive transfer of CD40L-expressing T cells, correlated with improved therapeutic efficacy in vivo.

Conclusion. Based on our results of the synergistic effect between Flt3L and CD40L on cDC1, we are now poised to further incorporate this into the Flt3L-secreting CAR T cell system to achieve cDC1 expansion and activation upon ACT in vivo. Our study has demonstrated an enhanced efficacy of CAR T cell treatment in solid cancers by harnessing the endogenous immune responses against tumours.

abs #164

New Method Development to Identify and Characterise Novel Targeted Breast and Ovarian Cancer Treatment

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Background. BRCA1/2-mutated cancer cells have a dysfunctional repair of damaged DNA, and the current advanced treatment used in the clinic for BRCA1/2-mutated cancer is poly-ADP ribose polymerase (PARP) inhibitor because it has a synthetic lethal relationship with the BRCA1/2 mutation, resulting in specific inhibition to the cancer cells. However, some BRCA1/2-deficient patients can develop PARP inhibitor resistance, and we need to find an alternative DNA repair pathway inhibitor that can selectively target BRCA1/2-mutated cancers. To achieve this, we need to develop a suitable screening method to identify and characterise new treatments for breast and ovarian cancers with BRCA1/2 mutations.

Methods/Results. Two cell lines were used in this study, which were BRCA1-negative cells and BRCA1-positive cells. These cell lines were grown separately for a traditional cytotoxicity assay, Sulforhodamine B (SRB) assay, and mixed for the newly developed method, called competitive growth assay, and the cells were treated with different concentrations of PARP inhibitor. In the SRB assay, the population of both cells was reduced in the presence of PARP inhibitor, with a higher number of cell survival in BRCA1-positive cells. The new method generated better results, in which the population of BRCA1-negative cells decreased as the concentration of PARP inhibitor increased, and the population of BRCA1-positive cells increased, meaning that the BRCA1-positive cells outgrowth the BRCA1-negative cells due to the drug targeting the BRCA1-negative cells.

Conclusion. Competitive growth assay is more sensitive and representative of tumour microenvironment than SRB assay, which can further help discover and characterise new targeted breast and ovarian cancer treatments.

abs #165

Interferon Regulatory Factors 1 and 2 regulate expression of Programmed Cell Death-Ligand 1 in dendritic cells

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Background. Programmed cell death-ligand 1 (PD-L1) is a regulatory molecule which is overexpressed in many cancers and plays a major role in supressing the immune system. Upregulation of PD-L1 on tumour-infiltrating dendritic cells (DCs) can lead to formation of an immunosuppressive environment which allows cancer cells to proliferate. Despite its importance, the regulation of PD-L1 in DCs remains largely uncharacterised.

Methods/Results. A genome-wide CRISPR/Cas9 screen searching for regulators of PD-L1 in DCs identified the transcription factor, interferon regulatory factor 2 (IRF2), as a promotor of PD-L1 cell surface expression. Production of a single-gene knockout of IRF2 in the MuTu DC line (Irf2-/-) allowed confirmation that Irf2-/- DCs have lower cell surface expression of PD-L1 when compared to wild type, without affecting the DC's ability to stimulate adaptive immunity. Reduced PD-L1 expression was also observed on Irf2-/- DCs following stimulation with toll-like receptor agonists, which are potent inducers of PD-L1 upregulation. In contrast, when Irf2-/- DCs were activated with interferon-□, a major driver of PD-L1 expression in the tumour microenvironment, there was no discernible difference in PD-L1 expression relative to wild type cells. Instead, the upregulation of another interferon regulatory factor, IRF1 is observed. In this scenario IRF1, a known promotor of PD-L1 in tumour cells, may drive PD-L1 upregulation independent of IRF2.

Conclusion. This study supports that in DCs, PD-L1 expression is regulated by the transcription factors IRF1 and IRF2, which play interchangeable roles dependent on the stimuli encountered in the microenvironment. Overall, these findings advance our understanding of the tumour immune response.

abs #171

Corona-CAR: Improving CAR T cell therapy using SARS-COV2 vaccination

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Background. Chimeric antigen receptor (CAR) T cells have revolutionised the treatment of blood cancers but fail to satisfactorily treat solid tumours. This involves isolating patient T cells and genetically modifying them to express a “CAR” that allows the recognition and killing of cancer cells. In addition to this CAR, T cells have their own T cell receptor (TCR) recognising an antigen. It has been shown that CAR T cell activity can be greatly enhanced when stimulating their TCR however this approach requires an effective T cell vaccine for clinical translation.

Following the COVID-19 pandemic, most people have either been infected with or vaccinated against the SARS-CoV-2 virus and therefore have SARS-CoV-2-specific memory T cells. Importantly, researchers have shown that the COVID-19 mRNA vaccines induce robust T cell responses, unlike many vaccines that predominantly induce antibody responses.

Methods/Results. We proposed that we could generate CAR T cells from these SARS-CoV-2 specific T cells (Corona-CAR) such that their anti-cancer activity can be boosted by administration of a COVID-19 mRNA vaccine. We have demonstrated that Corona-CAR T cells that were also stimulated with a COVID-19 mRNA had a greatly enhanced ability to kill cancer cell lines in vitro. Encouraged by the results, we injected Corona-CAR cells into mice bearing solid human tumours. Excitingly, the Corona-CAR T cells, when administered with a COVID-19 mRNA vaccine, eliminated the tumours.

Conclusion Using our “Corona-CAR” system, mRNA vaccines may be able to boost CAR T cell therapy to improve clinical outcomes for patients with solid tumours.

Cell Biology & Systems

abs #021

Regulation of thymocytes migration during T cell development by Coronin 2a, a putative actin-cytoskeleton regulator

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Background. T cell development occurs in the thymus and thymocytes follow a highly ordered migratory pattern through the organ as they develop. Regulation of the actin cytoskeleton is expected to control both cell movement and responses to extracellular signals. Coronin protein was selected because Coronin 1a (Coro1a) has been implicated in the regulation of mature T cell migration, activation, and trafficking process. The knockout of Coro1a, however, did not disturb T cell development. The knockout of Coronin 2a (Coro2a) affected the early stage of T-cell development and the migration of cancer cells.

Methods/Results. To investigate this, we developed a knockout mouse model where the coding region of Coro2a was replaced by a LacZ reporter. Using this reporter, we showed that Coro2a is upregulated during Double Negative (CD4-CD8-) stage and is then downregulated. Coro2a-/- mice significantly reduced thymus size and total thymocyte numbers. This decrease was due to an accumulation of thymocytes at the DN2 and DN3 stages with a subsequent loss of cells at the double positive (DP) stage. Analysis of mixed bone marrow chimera mice indicated that the effect of Coro2a deficiency is intrinsic to thymocytes. Coro2a-/- DN cells exhibited impaired migration towards certain chemokines in vitro. Confocal microscopy indicated that Coro2a co-localises with F-actin but is excluded from the cell's leading edge.

Conclusion. Together, this suggests that Coro2a may indeed be an important regulator of the actin cytoskeleton in DN cells, which in turn is important for their migration toward the thymic subcapsular zone. Further investigation aims to determine the precise molecular function of Coro2a within thymocytes.

abs #023

Deriving IBA1+ macrophages from human induced pluripotent stem cells

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Background. Objective: To derive IBA1+ macrophages from human induced pluripotent stem cells (iPSCs), characterised by immunocytochemistry and flow cytometry.

Methods/Results. Utilising growth factor-assisted embryoid body forming protocols, we optimised and generated iPSC-derived macrophages with reproducible and scalable results. Human iPSCs were cultured in low-adherence and growth factor-assisted conditions to induce mesodermal and hemogenic endothelium adaptation. Following this, cells were differentiated to a hematopoietic cell fate and myeloid specification using known myeloid-inducing cytokines. Myeloid-specified cells were terminally differentiated to produce macrophages.

Differentiated macrophages are characterised using a combination of known markers including CD14, CD68 and IBA1. Immunocytochemistry revealed approximately 90% positive expression for IBA1, whilst flow cytometry confirmed high expression of IBA1 (>99%). Other macrophage markers including CD68 and CD11 were also highly expressed at 63% and 51%, respectively.

Conclusion. We have generated an enriched population of IPSC-derived macrophages in vitro which display several characteristic markers. Future experimentation will be aimed at generating phenotypes consistent with macrophage populations in the cochlea, including CD36 and SPP1. These iPSC-derived macrophages could provide a viable model for investigating macrophage-mediated immune responses after cochlear implantation.

abs #028

Stem cell-derived macrophages in kidney organoids adopt a tissue-resident-like phenotype and associate with vasculature

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Background. Kidney organoids from human pluripotent stem cells (hPSC) have become a powerful model system for understanding human kidney disease and development. However, these applications are challenged by organoid immaturity. One possible factor limiting organoid maturation is the absence of tissue-resident macrophages. Animal studies have confirmed that tissue-resident macrophages exist in developing kidneys where they remove apoptotic cells and promote vasculature development. Therefore, we hypothesised that coculturing kidney organoids with hPSC-derived macrophages would promote the macrophages to adopt a tissue-resident phenotype, potentially improving organoid development by reducing apoptotic cells and improving organoid viability, as well as promoting organoid vasculature.

Methods/Results. We generated kidney organoids from hPSC through stepwise differentiation to kidney progenitors. We cultured two groups of organoids: with and without hPSC-derived macrophage addition (test and control). Then, we evaluated the differences between them. We found that after coculturing, the macrophages adopted a CD68+ tissue-resident macrophage-like phenotype, with significantly increased proportions of CD14+CD68+ macrophages in the test organoids compared to the macrophages culture alone where they were CD14+CD68−. The macrophages also significantly lowered early apoptotic cell proportions in the test organoids compared to the control. Furthermore, we observed physical interactions between the macrophages and vasculature, suggestive of a role in organoid vasculature development.

Conclusion. In summary, we demonstrate that cocultured hPSC-derived macrophages develop a tissue-resident-like phenotype, remove apoptotic organoid cells, and associate with vasculature. Further analyses will determine whether macrophages can enhance the proportion, complexity, and maturation of the vasculature within kidney organoids, improving their suitability as a model for disease and development.

abs #036

The role of O-GlcNAcylation in the regulation of dendritic cell function and immunity

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Background. O-GlcNAcylation is a dynamic post-translational modification of protein characterized by the mono-glycosylation of a terminal β-N-acetylglucosamine (GlcNAc) moiety to serine and threonine residues of nucleocytoplasmic and mitochondrial proteins. Protein O-GlcNAcylation is involved in many crucial cellular processes including transcription, translation, epigenetics, cell signaling, and development. Homeostatic control of protein O-GlcNAcylation is essential for cell physiology; dysfunctional O-GlcNAcylation has been implicated in various pathologies.

O-GlcNAc cycling is governed by the reciprocal activity of two enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) that catalyzes the attachment and removal of O-GlcNAc respectively.The critical immunoregulatory roles of O-GlcNAcylation have been explored in various innate and adaptive immune cells. For examples, OGT ablation results in enhanced apoptosis of germinal centre B cells and memory B cells in an immune response, and OGT deficiency in T cells leads to an impaired activation of transcription factors such as NFAT and NFκB. However, little is known about the role of O-GlcNAcylation in regulating dendritic cell development and function.

Methods/Results. Here, we developed a DC-specific Ogt conditional knockout mouse model using the Cre/loxP strategy to investigate the role of OGT in modulating dendritic cell development, cell turnover, maturation, and activation. Mice with a CD11c-specific ablation of Ogt exhibit defective splenic cDC homeostasis, characterized by a specific reduction in of the cDC2A (ESAMhiClec12a-) population. In addition, OGT-deficient ESAMhiClec12a- cDC2A exhibited an activated phenotype even at steady state.

Conclusion. Our ongoing data demonstrate the importance of OGT in regulating dendritic cell function and homeostasis.

abs #037

Are Oligodendrocytes an Under-Appreciated Therapeutic Target in ALS?

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Background. Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterised by the degeneration of motor neurons. However, the surrounding glia, including oligodendrocytes also exhibit ALS pathology, suggesting an undiscovered and underappreciated role in disease. If oligodendrocytes and myelin are intrinsically dysfunctional, could they provide a therapeutic target? To address this, we have extensively characterised oligodendrocyte dysfunction and altered myelin behaviours in the clinically relevant TDP-43Q331K ALS mouse.

Methods/Results. TDP-43Q331K mice (ALS; n=3-6) and wildtype littermates (WT; n=3-6) were collected pre-symptomatically, symptomatically, and at end-stage. Using a combination of EdU and BrdU, oligodendroglial cell density, proliferation, and differentiation were tracked over time in the dorsal column, ventral white matter, and ventral grey matter of the lumbar spinal cord. Myelin was examined using a combination of Spectral Confocal Reflectance Microscopy and electron microscopy (EM).

In end-stage mice there were significant increases in oligodendrocyte precursor cell (OPC) density, and oligodendrocyte proliferation and differentiation in the ventral grey matter in the ALS mice compared to WT ($p<0.05$). Both SCoRe and EM revealed that myelin density was significantly increased in the dorsal column of ALS mice ($p<0.05$). No significant changes were found pre-symptomatically or during early symptom onset.

Conclusion. We are the first to characterise the oligodendrocyte lineage and myelin in a TDP-43 related ALS mice. Our data suggest that OPCs and oligodendrocytes display altered pathology in their proliferation and myelin generation, suggesting that they are indeed impacted in ALS and garner further exploration into their potential use as a therapeutic target.

abs #041

Characterising the function of the mitochondrial protein DNAJC15 at the TIM23 complex

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Background. To produce energy for our cells, mitochondria rely on the function of over 1,300 mitochondrial proteins. The majority of these mitochondrial proteins must be transported into mitochondria from the cytosol. This transport process is facilitated by large machineries known as translocases. My project is focused on the TIM23 complex, a translocase made up of many protein parts. One of these protein components is DNAJC15, which has been linked to breast and ovarian cancer. Despite identification of DNAJC15 as a TIM23 component, little is known about its function in mitochondria. My project aims to characterise the function of DNAJC15 in mitochondrial biogenesis.

Methods/Results. Using a DNAJC15 knock-out cell line (generated in honours), label free quantitative proteomics was performed to determine the impact of DNAJC15 depletion on the mitochondrial proteome. We identified limited statistically significant changes following depletion of DNAJC15 in HEK293 cells. Re-expression of flag-tagged constructs in the knock-out background were used in co-immunoprecipitation experiments. These studies demonstrated DNAJC15 interacts with TIM23 components MAGMAS and TIM50 and protein import and sorting factors such as STOML2 and the prohibitins. Interestingly, DNAJC15 interacts with mitochondrial inner membrane quality control factors such as mitochondrial proteases.

Conclusion. From the co-immunoprecipitation data and pre-existing literature, DNAJC15 appears to be involved at the TIM23 complex but may have novel roles in other mitochondrial pathways. The mitochondrial interactome suggests DNAJC15 may be involved in mitochondrial quality control. In association with the literature, the lack of proteomic changes following knockout of DNAJC15 suggest DNAJC15 may have tissue specific functions.

abs #083

Pathogenic variants in mitochondrial protein import subunit TIM50 impact respiratory complexes and proteome constitution

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Background. The import of cytosolically synthesised proteins into mitochondria is a highly regulated process that is vital for organelle function. Protein translocases embedded within the mitochondrial membranes are responsible for both the physical movement of proteins and their correct sub organellar localisation. Mutations in subunits of the TIM23 complex, an inner mitochondrial membrane translocase, are increasingly being connected to rare genetic mitochondrial disease.

Methods/Results. Here we describe a mitochondrial disease patient that possesses a novel mutation in the TIM23 complex subunit, TIMM50. The function of TIMM50 in mammalian mitochondrial protein import is poorly explored and the pathogenic connection between TIMM50 and mitochondrial disease remains unclear. Quantitative mitochondrial proteomics and native-gel analysis revealed that loss of TIMM50 in patient fibroblasts drastically reduced the levels of endogenous TIM23 complex. In-vitro import experiments show reduced protein import kinetics for TIM23 cargo proteins in patient fibroblasts. This significantly alters the mitochondrial proteome, with impactful reductions in certain oxidative phosphorylation (OXPHOS) complexes and changes to mitochondrial ultrastructure. These defects are highly specific and cannot be adequately explained with our current understanding of mitochondrial protein import.

Conclusion. Our data suggests that different mitochondrial pathways have unique sensitivities to defects in protein import, extending to specific OXPHOS complexes. Extensive proteomic analysis has enabled the identification of specific TIM23 complex functions that have increased dependence on TIMM50. This work has given significant insight into the role of protein import in mitochondrial disease, identifying potential hotspots of pathogenicity and alluding to novel functions for TIMM50 beyond its presumed roles in protein import.

abs #092

In-vitro cytotoxicity of direct 3-dimensional printed aligners

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Background. Three-dimensional (3D) printing technology is an innovative method for manufacturing aligners. This study aimed to compare the in-vitro cytotoxicity of 3D-printed aligners with conventionally thermoformed aligners (TFA) under different post-curing conditions.

Methods/Results. Three materials were evaluated: 3D-printed resin (TC-85DAC, Graphy, Korea), thermoformed Smart Track (Align Technology, USA), and thermoformed TruGEN (Spark, Ormco, USA). Three different post-curing conditions were assessed: with Nitrogen for 14 minutes (P1), without Nitrogen for 14 minutes (P2), without Nitrogen for 30 minutes (P3).
Aligners were cut in smaller specimens (2mmx2mm) and sterilized at 121°C. The samples were placed at 37° in 96-well plates containing Dulbecco's Modified Eagle's Medium (DMEM) for 7 and 14 days. The cells viability of primary human dental bud stem cells (DBSCs) cultured with DMEM was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Optical density values obtained from cell cultures were normalized to untreated controls, summarized as means and statistically analyzed (alpha=0.05).
The cytotoxicity comparison revealed no statistically significant differences between DPA (P1) and TFA ($P<0.001$). The P1 procedures significantly improved the cytocompatibility of DPA compared to the P2 ($P<0.001$) and P3 conditions ($P<0.01$). Overall, the P1 process showed a high cytocompatibility at each timepoint, with no significant reduction between 7 to 14 days. On the contrary, while both the P1 and P2 conditions displayed high biocompatibility after 7 days, their cell survival significantly reduced over time (P2, $P<0.001$; P3, $P<0.01$).

Conclusion. 3D-printed aligners may be a valid biocompatible alternative to traditional thermoformed aligners. However, the post-curing processes may significantly affect the DPA cytotoxicity.

abs #102

Shedding light, and electrons, on MLKL oligomerisation within native cell membranes

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Background. Mixed Lineage Kinase domain-Like (MLKL) protein is the terminal effector of necroptotic cell death. MLKL oligomers accumulate at the cell membrane where it contributes to membrane permeabilization and cell lysis. Several conflicting models have been proposed on the mechanism which cell permeabilization occurs. Whether lytic function relies on direct embedment of MLKL's N-terminal four-helix bundle (4HB) domain within the plasma membrane is still unclear. Previous structural studies indicate that the 4HB is unleashed and released from MLKL's brace helices, which is triggered upon conformational changes within the pseudokinase domain. The orientation of the MLKL's 4HB, brace helix and pseudokinase domains during membrane engagement is still unresolved.

Methods/Results. Here we propose to determine the stoichiometry and domain topology of MLKL oligomers post-necroptotic stimuli within native membranes of giant plasma membrane vesicles (GPMVs) by cryo-electron tomography (Cryo-ET). The stoichiometry of MLKL oligomers will also be investigated in in-tact cells by single-molecule localisation microscopy – point accumulation for imaging in nanoscale topography (PAINT).

Conclusion. These studies will illuminate how MLKL oligomers assemble and perforate membranes to execute cell death.

abs #112

USP16: A cellular ubiquitin rheostat?

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Background. Ubiquitin (Ub) exists in a dynamic equilibrium between its attachment to substrates and the free mono form. Proteostasis adapts swiftly to changes in these pool dynamics, suggesting regulation by the Ub pool. We propose the existence of a rheostat capable of both affecting and sensing Ub pool dynamics, as well as possessing intrinsic comparator/setpoint properties.
USP16, a deubiquitinase, can remove Ub from substrates (affector) and contains a Ub C-terminal binding Zinc Finger (Znf) UBP domain (sensor), potentially allowing free Ub to regulate the enzyme's activity through kinetic or allosteric effects (comparator/setpoint). USP16 also deubiquitinates histone H2A K119 and ribosomal subunits, suggesting a role in gene regulation. USP16's biochemical properties and physiological function make it a logical candidate for a Ub rheostat.

Methods/Results. Fluoresence polarization showed he Znf-UBP domain bound mono Ub at 10 μM, and a 1.8 Å crystal structure of the domain allowed design of a non-binding mutant. Fluorescence-based kinetics assays of full-length variants showed significantly reduced activity in the mutants. Surface Plasmon Resonance showed catalytically blocked Full-Length USP16 showed the Znf-UBP domain binds Ub independently of catalysis, whilst an engineered Ub that only binds the Znf-UBP appears to inhibit the reaction. Finally, Mass Photometry was used to determine that USP16 and the Nucleosome form a stable complex.

Conclusion. The Znf-UBP domain potentially regulates USP16's activity, enabling free Ubiquitin to enter and subsequently inhibit catalytic turnover. This may further serve as a mechanism for regulating substrate interaction, such as with the Nucleosome to be investigated using Cryo-EM.

abs #114

Structural studies investigating the binding of a novel inhibitory peptide to the β-common receptor

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Background. The β-common receptor (βCR) is the shared receptor subunit for the cytokines interleukin (IL)-3, IL-5 and GM-CSF. Receptor subunits for these cytokines are members of the haematopoietin receptor superfamily. βCR can also interact with the erythropoietin (EPO) receptor to form the innate repair receptor (IRR). Activation of the IRR is associated with an increase in adverse cardiovascular events. Recently, a novel βCR inhibitory peptide (βIP) was designed based on an α-helix in EPO and reported to inhibit nitric oxide production and the angiogenic potential of the IRR, without affecting erythropoiesis. How βIP interacts with the βCR, and the structure of the complex, are yet to be established. Therefore, my project is to determine the binding kinetics between βIP and βCR, along with the structure of their complex, in order to clarify the interactions between them and provide guidance for further inhibitor design.

Methods/Results. βCR was expressed in insect sf21 cells, purified via Ni-IMAC and size exclusion chromatography, and characterised by tryptic digest mass spectrometry (MS), LC-MS, mass photometry, thermal melt assay, circular dichroism and differential scanning fluorimetry to confirm its quality. The peptide was characterised by MS, HPLC and dynamic light scattering, thus confirming its pure and monomeric state. In a direct binding assay (surface plasmon resonance), βIP did not demonstrate any measurable binding to βCR. Orthogonal direct binding assays will now be conducted (microscale thermophoresis, hydrogen-deuterium-MS) to establish whether βIP interacts with βCR or to confirm the surface plasmon resonance results.

Conclusion. My results to date suggest that βIP is not exerting its biological function through direct interaction with the βCR.

abs #118

Functional characterisation of the human mitochondrial disaggregase, CLPB

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Background. Human CLPB is a mitochondrial, AAA+ domain containing protein with demonstrable disaggregase activity in-vitro. Pathogenic mutation of CLPB most frequently manifests in 3-methylglutaconic aciduria with associated neuropathies and neutropenia (MGCA7A/B), though cases of severe congenital neutropenia (SCN9) alone have also been reported. We and others have shown that CLPB interacts strongly with HAX1, a largely uncharacterised and highly disordered intermembrane space resident. CLPB also has purported interactivity with components of the inner membrane proteolytic SPY complex (STOML2, PARL and YME1L1), which may be indicative of novel cooperative proteolytic action. It is currently unknown if such specific interactions have broader functional significance.

Methods/Results. To explore this possibility, we have established a CLPB knock-out (KO) cell line into which we have stably introduced wild type and substrate trap mutant CLPB. Via LFQ quantitative proteomics we report a consistent phenotype between our KO model and MGCA7B patient lymphoblasts and utilise our substrate trap mutants to succinctly define the human CLPB interactome. To probe CLPB disaggregase function, we examine the capacity of both wild type and mutant CLPB to clear basal and stress-induced aggregation within the KO through both proteomic and fixed-cell imaging techniques.

Conclusion. By coupling immunoprecipitation and aggregation propensity data we define a discrete list of human CLPB substrates, including HAX1, HTRA2, OPA1 and all three SPY complex members. Our work clarifies the specificity of CLPB disaggregase action, implicates CLPB as a supportive component of various IMS systems and establishes how associated disease heterogeneity may be partly attributed to protein quality control network interconnectivity.

abs #120

Manipulation of mitochondrial functions by Legionella pneumophila

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Background. Legionella pneumophila, a waterborne opportunistic pathogen causing community-acquired pneumonia termed Legionnaires' disease, establishes an intracellular replicative niche within human alveolar macrophages via its type IVB secretion system (T4SS). Over 330 Legionella effector proteins are translocated into the host cells by T4SS to manipulate host functions, including mitochondrial functions. However, only a handful of effectors are currently known to target mitochondrial functions, primarily affecting mitochondrial dynamics and ATP transport across the inner mitochondrial membrane.

Methods/Results. We used label-free quantitative mass spectrometry on mitochondria purified from infected THP-1 cells, identifying T4SS effector proteins enriched in mitochondria. Using immunofluorescence microscopy, four effectors were found localised to mitochondria when ectopically expressed. Three show C-tail anchored (TA) protein characteristics, two of which were validated to localise to the outer mitochondrial membrane (OMM) through sub-mitochondrial fractionation and carbonate extraction. Mutagenesis and immunofluorescence microscopy verified the essential role of TMD and C-tail for one effector mitochondrial localisation. These are the first identified Legionella T4SS effectors targeting OMM, raising the possibility that Legionella could use TA effectors for OMM targeting. To answer this, we screened 275 experimentally validated T4SS effectors for those that contain a single transmembrane domain (TMD) using InterPro, TMHMM, and TOPCONS. Nine have their TMD within ~30 residues of the C-terminus and a net positive C-tail charge, thus are potential mitochondrial TA effectors. Immunoprecipitation and mass spectrometry are then used to characterise these effectors.

Conclusion. Our findings suggest that L. pneumophila could exploit C-tail anchored effectors to target OMM, thereby modulating mitochondrial functions during the infection.

abs #122

Meox1 is a transcriptional effector of Hippo signalling that controls endothelial cell size during embryonic venous sprouting and lymphangiogenesis

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Background. Lymphatic vascular development involves the specification of lymphatic endothelial progenitors from venous endothelial cells that subsequently undergo sprouting, proliferation and cell growth. Despite recent developments in the field, regulation of this enigmatic developmental process at a cellular and molecular level, remains to be fully understood. The Hippo pathway and its effectors, Yap and Taz, control organ growth and morphogenesis. Previous work done by the lab found that YAP displays dynamic changes in subcellular localisation in lymphatic progenitors during Vegfc-dependent sprouting, and is indispensable for lymphatic vascular development in zebrafish. Interestingly, the blood vasculature of these Maternal and Zygotic (MZ) yap1 mutants was found to be normal.

Methods/Results. In this study, we have utilised single-cell RNA and ATAC-sequencing of whole embryonic vasculature dissociated from MZ yap1 mutants to isolate venous cells consisting of fated lymphatic progenitors. A differential gene expression analysis in these cells identified downstream transcriptional target genes of Yap, including meox1, as an unexpected, tissue specific, and functionally essential regulator of lymphatic development. In vivo analysis also revealed a transcriptional relationship between Hippo signalling and meox1 in the developing vein of the zebrafish embryo.

Conclusion. Together, we propose Vegfc signalling to regulate Yap, thereby controlling meox1 transcription and sprouting of venous cells. This work uncovers a unique and previously unappreciated transcription factor, meox1 to control venous sprouting, independent of cell fate control.

abs #128

Understanding the interaction of KLHDC3 with the RECQL4 mutations observed in Rothmund-Thomson Syndrome

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Background. RECQL4 belongs to a conserved family of RecQ helicases, functioning in double-strand break repair and initiation of DNA replication. Mutations in the gene encoding this protein are associated with diseases including Rothmund-Thomson Syndrome, a rare disease characterised by poikiloderma, skeletal abnormalities, and increased predisposition to osteosarcoma.

Methods/Results. A genome-wide loss of function rescue screen was performed in mouse cells to identify genetic modifiers of the effects of mutant RECQL4, and the loss of Klhdc3 was identified as rescuing RECQL4-deficient cells. KLHDC3 participates in destruction via C-end degrons pathway, leading to ubiquitination and protein degradation. It is hypothesised that the most likely mechanistic basis for the rescue is that proteins normally targeted by KLHDC3 functionally substitute for RECQL4 in the initiation of DNA replication.
We aimed to determine if the knockout of possible key substrates through specific depletion by sgRNAs can potentially reverse the rescue in Recql4-/- sgKlhdc3-/- cells and lead to synthetic lethality.
Candidate genes were identified from another loss of function screen – the top hit of which was Cyclin F. Similarly, CCNF functions in ubiquitination and degradation. We confirmed the synthetic lethality of CCNF as shown by a decrease in proliferation of sgCcnf-targeted cells and a rapid depletion of Ccnf knockout cells by day 4.

Conclusion. These results suggest a possible link between CCNFF expression and mediating protection from the loss of RECQL4-initiated DNA replication. Further understanding of RECQL4 mutations, particularly of the pathways that allow for the modification of the function of the affected genes, can help elucidate novel approaches.

abs #144

CRISPR-Cas13e knockdown of VEGFA mRNA for treatment of ocular neovascularisation

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Background. New CRISPR-Cas13 proteins allow for precise RNA editing, an emerging strategy for safer gene therapies. RNA editing can achieve therapeutic effects in a safer, reversible, and flexible manner, whilst being delivered from a single adeno-associated virus (AAV) vector. We hypothesised that RNA silencing of vascular endothelial growth factor A (VEGFA) using the new Cas13e enzyme would control ocular neovascularisation.

Methods/Results. HEK293FT and ARPE19 cells were transfected with Cas13e carrying either a non-targeting (NT) guide RNA (gRNA), VEGFA targeting gRNA or an array of three VEGFA targeting gRNAs. VEGFA mRNA expression was quantified using RT-qPCR, and off-targets were identified from RNA sequencing. Up to 90% VEGFA mRNA knockdown was observed with single and array of VEGFA targeting gRNAs, compared to NT sgRNA controls. No off-target effects were observed with single guide RNA, while one other gene, IFIT2, was significantly downregulated with an array of gRNAs in a guide-independent manner. The constructs were then packaged into AAV2Max vectors and intravitreally injected into Kimba mice expressing human VEGFA. Eight weeks later, mice eyes were imaged using fundus fluorescence angiography and vessel densities analysed using AngioTool. VEGFA mRNA expression was similarly quantified. Moderate knockdown (≤20%) of VEGFA mRNA was observed with Cas13e carrying an array of gRNAs along with significant reduction in vessel density, suggesting control of neovascularisation.

Conclusion. Efficient and specific knockdown of VEGFA mRNA can be achieved in mammalian cells using single-AAV plasmids, demonstrating proof-of-concept for long-term Cas13e-mediated anti-VEGF therapy. Phenotypic improvement in mice suggests clinical potential of Cas13e for treating neovascular ocular diseases.

abs #148

Lost in translation: RNF14 tags translation factors during ribosomal stalling to prevent dysfunctional protein synthesis

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Background. Proteins, the workhorses of the cell, orchestrate all cellular processes from division to death. To perform these critical roles and prevent pathological functions, optimal protein levels are achieved by balancing synthesis by ribosomes and degradation by proteasomes. Ribosomal protein synthesis is tightly regulated to ensure the generation of fully functional proteins. Ribosomal quality control (RQC) pathways identify and respond to disruptions in protein translation, such as ribosomal stalling. Such stalling on the mRNA recruits specific E3 ubiquitin ligases to 'tag' ribosomal components and release the stalled ribosome. This prevents the synthesis of dysfunctional protein products, which are often associated with diseases including Cystic Fibrosis and Hurler Syndrome. The understudied E3 ubiquitin ligase, RNF14, is implicated in a novel RQC pathway where it induces degradation of translation factors associated with stalled ribosomes [1]. However, the mechanism of how RNF14 tags these factors, and the resulting downstream signalling remains enigmatic.

Methods/Results. To address these shortcomings, we've used a combination of in vitro reconstituted systems, structural biology, mass spectrometry and cell-based assays to show that, upon inducing ribosomal stalling by specific drugs, RNF14 generates unconventional, branched ubiquitin chains in vitro and in cells. Given this unusual specificity we are now investigating the mechanism by which RNF14 regulates RQC pathways and its downstream signalling functions.

Conclusion. RNF14 regulates a novel RQC pathway by modifying the translational machinery with unconventional ubiquitin tags. Understanding the mechanism behind these events will provide insight into the pathogenesis of diseases caused by truncated proteins and may lead to novel therapeutic options.

abs #150

Redefining Transplants: Bioengineering the Corneal Endothelium using Human Stem Cells & a Hydrogel Film

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Background. In response to the worldwide deficit in tissue for corneal transplantation, our objective was to fabricate a fully bioengineered corneal endothelium. The challenge was sourcing corneal endothelial cells (CECs) due to their scarcity and finding a suitable biodegradable material to carry them.

Methods/Results. We developed a differentiation protocol (n=4), transforming induced pluripotent stem cells (iPSCs) into iPSC-derived corneal endothelial-like cells (iCECs) within 10 days. iPSCs were cultivated in tissue culture plastic (TCP) coated with either vitronectin (VTN) or laminin 511 (L511), then maintained daily with E8-Flex medium (3 days). Differentiation employed basal induction medium enhanced with: SB431542 and Noggin for neural crest cell induction (2 days); and PDGF-BB, DKK-2, and B27 for iCEC induction (8 days).
Characterisation was undertaken by RT-qPCR, immunocytochemistry, and morphometric analysis. Both coatings displayed reduced OCT4 pluripotency marker expression. Although Nestin, a neural marker, was consistently observed, areas of its absence were noted in VTN-coated cultures. ZO 1, a conventional CEC marker, was surprisingly consistently present and serendipitously assisted in tracking morphological shifts. Importantly, L511-coated cultures exhibited smaller polygonal cells similar to CECs. Additionally, passaging iCECs led to further reduction of OCT4 and Nestin levels.
Finally, we successfully integrated iCECs onto an RGD-functionalised polyethylene glycol (PEG)-based hydrogel film (n=4), thus producing a bioengineered corneal endothelium.

Conclusion. In light of the global shortage of corneal tissue, our bioengineered corneal endothelium presents a promising alternative for artificial grafts, potentially revolutionising treatments to restore vision in patients with corneal endothelial diseases.

Child, Women and Reproductive Health

abs #005

Therapeutic Effects of Topical Cetirizine in Treatment of Female Pattern Hair Loss: A Randomized Controlled Noninferiority Trial

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Background. Female pattern hair loss (FPHL) is the most prevalent form of hair loss in women. It was aimed to evaluate the therapeutic effects of topical cetirizine 1%, versus topical minoxidil 2% in patients with FPHL.

Methods/Results. Through a six-month triple-blind randomized clinical trial, 60 women with FPHL, were randomly divided into two groups of treatment with topical cetirizine 1% or topical minoxidil 2%. The endpoint was changing in hair loss severity as well as terminal hair density and diameter, according to trichoscopic evaluation. Intention-to-treat analysis was also performed for those who accomplished three months of treatment.

Both groups showed improvement in hair diameter and density after six-month; however, the outcome was significant only in the minoxidil group. According to per-protocol analysis, minoxidil was significantly superior to cetirizine in hair density, but not in hair diameter. According to the hair loss severity scales, FPHL was significantly improved in both groups after six months. The dropout rate due to adverse effects was 10.0% and 6.6% in the cetirizine and minoxidil groups, respectively.

Conclusion. Although inferior to topical minoxidil, topical cetirizine can provide favorable therapeutic effects for FPHL, specifically when patient incompatibility with minoxidil is observed.

Trial registration: The study is registered in the Iranian Registry of Clinical Trials (IRCT) with the registration code IRCT20200521047536N1

abs #034

Relationship between teacher-rated listening ability and auditory processing, speech, hearing, and cognitive abilities in primary school children.

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Background. Children are often referred to health professionals with concerns of listening difficulties despite having normal hearing thresholds. The diagnosis made can be influenced by the referral pathway – an audiologist may diagnose the child as having auditory processing disorders, a speech therapist may identify a speech and language disorder, or a psychologist may determine attentional, or memory deficits are present.
There is a growing body of literature supporting listening difficulties being due to a complex range of deficits that differ in combination for each child including deficits in auditory processing, cognition, speech sound perception, language, and hearing deficits. If undiagnosed, these deficit/s can impact the learning outcomes of children. Appropriate diagnosis of the underlying deficit will allow for targeted rehabilitation approaches.

Methods/Results. A tri-level test battery is being developed that assesses the contribution of auditory processing, speech sound perception, language, cognition, and hearing to real-life outcome measures for primary school aged children (aged 6-11 years). These outcomes are obtained in the form of questionnaire-based ratings, listening tests and reading tests.
This research uses regression analysis to explore how the underlying abilities relate to teacher-rated listening ability in the classroom.

Conclusion. From the data collected till date, a strong correlation can be established between the high level test and measures used for receptive language within the test battery. A significant correlation between the high level test and the teacher rated listening ability indicate that teacher's are highly adept to predicting possible listening difficulties in these children.

abs #038

IVF Success Rate Rates in Individuals Accessing Preimplantation Genetic Testing for Monogenic Conditions (PGT-M) in Victoria, Australia

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Background. Pre-implantation genetic testing for monogenic conditions (PGT-M) is a growing indication for IVF in Australia. However, there are no studies reporting clinical outcomes in an Australian population. The aim of the current study is to analyse clinical outcomes of PGT-M at Monash IVF, one of the Victoria's largest providers.

Methods/Results. Clinical outcomes of embryos subjected to PGT-M from 2015-2022 were extracted from the clinical database. Simultaneous monogenic and cytogenetic analysis using a karyomapping protocol was the standard during this period. Descriptive statistics were analysed using STATA v.14.2.

Between January 2015 and August 2022 Monash IVF's Victorian laboratories tested 2344 embryos for monogenic indications, from 527 stimulated cycles. 798 (34%) of embryos analysed were both euploid and low probability of the condition of interest and considered suitable for transfer, while 1217 (52%) were either aneuploid (n=372) or high probability of the condition of interest (n=845) and considered not suitable for transfer. 512 embryos were subsequently transferred, resulting in 263 clinical pregnancies, and 231 live births and ongoing pregnancies. The clinical pregnancy and birth rates per embryo transfer were 51.4% [95% CI: 47.0-55.7%] and 45.1% [95% CI: 40.9-49.5%] respectively. Clinical pregnancy and birth rate per cycle resulting in embryo biopsy were 49.9% [95% CI: 45.7-54.2%] and 43.8% [95% CI: 39.7-48.1%].

Conclusion. Couples accessing IVF for PGT-M have higher clinical pregnancy and live birth rates than couples accessing IVF for other indications such as infertility. These findings confirm that PGT-M is an effective reproductive option for Australian carrier couples.

abs #084

Impact of intrapartum azithromycin on macrolide resistance in Escherichia coli isolated from infants

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Background. Neonatal sepsis, a leading cause of morbidity and mortality in sub-Saharan Africa, necessitates context-specific interventions. Azithromycin given during labour, a novel intervention with potential to decrease maternal and neonatal infections, decreased colonization with gram-positive bacteria with a transient increase in macrolide resistance. There is limited data on the intervention's effect on Escherichia coli, a major gram-negative bacterium causing neonatal sepsis. We aim to determine the effect of 2g of oral intrapartum azithromycin on carriage of macrolide resistance determinants and genetic diversity of E. coli from a subset of infants whose mothers participated in a double-blind clinical trial (PregnAnZI-2) conducted in The Gambia and Burkina Faso between October 2017 and May 2021.

Methods/Results. Here we included 311 infants of mothers in PregnAnZI-2 (152 received azithromycin, 159 received placebo). Using whole genome sequencing we determined carriage of macrolide resistance genes (mph, erm, ere, msr) and genetic diversity of 396 E. coli isolated from these infants' rectal swabs, collected at Day 6 and Month 4 post-intervention. Results show a broad multi-locus sequence type diversity of E. coli from infants in both trial arms. There was higher prevalence of E. coli carrying macrolide resistance genes at Day 6 (29.9% versus 12.1% p=0.004) and no significant differences at Month 4 (28.6% versus 19.6% p=0.170) in the azithromycin arm compared to placebo.

Conclusion. These data, suggest the intervention could pose a risk of macrolide resistance emergence in the community, underscoring the need for continuous monitoring of macrolide resistance and transmissibility of associated genes during such interventions.

abs #085

Functional Characterisation of SRY Gene Variants in Patients with 46, XY Differences of Sex Development (DSD)

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Background. In mammals, male sexual development is a complex and precise process initiated by the expression of the Sex-determining Region of Y (SRY) gene. The intronless SRY encodes a 204 amino acid protein containing a highly conserved High Mobility Group (HMG) box flanked by two nuclear localization signals (NLSs) domains termed CaM NLS and β NLS. The SRY protein is a transcription factor that binds DNA and activates genes involved in the testis development pathway. Variations in SRY are known to cause Differences of Sex Development (DSD) and account for 10-15% of 46, XY gonadal dysgenesis cases.

Methods/Results. In this study, we report the functional analysis of four SRY variants we have previously identified using massively parallel sequencing in patients with 46, XY DSD. All variants (p.Asp58Glu, p.Arg75Lys, p.Met85Thr, and p.Arg86Term) are located inside the HMG box. We present preliminary data showing the impact of these variations on the normal function of the SRY protein. In silico analysis revealed that all missense variants affect the flexibility of the SRY protein which may impair its DNA binding activity. We also examined the localization of the SRY variants using a Flag tagged-SRY fused protein and show that two variants (p.Arg75Lys and p.Met85Thr) affect the nuclear import of SRY. The truncating variant, p.Arg86Term abolishes SRY expression.

Conclusion. Our data confirm the likely pathogenicity of these variants and improve diagnostic certainty for the patients in which they were identified.

abs #097

The effect of being born moderate-late preterm on lung function during school-aged years

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Background. The detrimental effect of birth at <32 weeks' gestation on lung function is well established. However, the effect of moderate-late preterm (32 to 36 completed weeks' gestation; MLP) birth on lung function during childhood remains unclear.

Aim: To assess the effect of being born MLP, compared with being born at term, on lung function at 9 years of age.

Methods/Results. Prospective cohorts of children born either a) MLP or b) at term at the Royal Women's Hospital, Victoria, Australia. Participants completed pre- and post-bronchodilator spirometry, single breath diffusing capacity for carbon monoxide [DLCO], whole-body plethysmography and nitrogen multiple breath washout at 9 years of age. Mean differences [MD] in z-scores between those born MLP and at term were estimated using linear regression models with adjustment for potential confounding.

148 children born MLP and 109 term controls were assessed. Compared with controls, MLP children had lower z-scores (MD, 95% confidence interval) for forced expiratory volume in 1 second [FEV1]: -0.39, (-0.65, -0.13), FEV1/forced vital capacity [FVC]: -0.41, (-0.66, -0.16), forced expiratory flow [FEF25-75%]: -0.38, (-0.64, -0.11) and DLCO: -0.23, (-0.43, -0.02). Similar z-scores were observed for FVC: -0.2, (-0.46, 0.07), total lung capacity [TLC]: -0.13, (-0.33, 0.08), residual volume [RV]: -0.01, (-0.1, 0.09), RV/TLC: -0.01, (-0.1, 0.09) and lung clearance index [LCI2.5%]: -0.08, (-0.46, 0.29) between birth groups.

Conclusion. Reductions in expiratory airflows and diffusion capacity in the first decade after birth occur in children born MLP, which may predispose them to later chronic obstructive lung disease.

abs #100

High throughput functional genomics – the next big hit

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Background. Genetic conditions are a major contributor to death in childhood. Approximately 400 million individuals are affected by rare diseases globally. Application of next generation sequencing to children with rare diseases has yielded diagnostic rates of up to ~60%. Despite this great success, nearly half of children remain undiagnosed. One of the major contributors is the absence of functional evidence to experimentally confirm the pathogenicity of a potentially relevant variant. The broad range of functional assays and the vast number of causative genes reported for monogenic conditions are barriers to generating functional evidence in an efficient and robust manner. This time-consuming and costly process could be alleviated by the availability of a validated high-throughput functional assay.

Mass spectrometry-based proteomics has received growing attention in the last two decades due to its large-scale and high-throughput nature. Proteomics has been used extensively in research settings, including biomarker discovery and protein profiling during functional genomics studies. More recently it has started taking centre stage in providing functional solutions to validation of pathogenicity in rare disease diagnosis, however validation of proteomics as an orthogonal clinical tool for rare disease diagnosis is still ongoing.

Methods/Results. We aim to establish the clinical utility of proteomics as a universal assay under a range of conditions. In this presentation, we will report on our analyses of published literature where proteomics was used, providing evidence to demonstrate the practical applications of the technique across all inheritance patterns and for all common variant types.

Conclusion. It has been demonstrated from the literature and examples from this study that Mass spectrometry-based proteomics holds a great potential in delivering genetic diagnosis to families in need.

abs #103

WD-repeat containing protein-61 dysregulation in the endometrial luminal epithelium impairs human endometrial receptivity

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Background. Endometrial receptivity (ER) is a hallmark of successful blastocyst implantation in early pregnancy. A major challenge to successful IVF treatment is recurrent implantation failure (RIF) whereby impaired ER is a major contributor. However, there is no way to identify or treat abnormalities in the endometrium. Recently, we identified WD-repeat-containing protein-61 (WDR61) was abnormally reduced in apical endometrial epithelial organoid secretions from primary infertile women (N=7). WDR61 is a transcriptional cofactor of the Wnt pathway, which has known roles in endometrial function. We hypothesised that WDR61 plays a role in ER during the window of implantation (WOI).

Methods/Results. Immunohistochemistry demonstrated WDR61 in endometrial glands, stroma, and luminal epithelium (N=7). WDR61 was significantly higher during the receptive phase than in the non-receptive, proliferative phase of fertile women (N=8, $P<0.05$). To assess function, siRNA knockdown of WDR61 in Ishikawa (endometrial epithelial cell-line) and primary human endometrial epithelial cells were used to assess adhesion by xCELLigence real-time monitoring and trophoblast spheroid adhesion. WDR61 siRNA treatment reduced adhesion compared to respective controls ($P<0.05$). Several pivotal ER genes such as HOXD10, MMP2, CD44 and CXCR4 were significantly diminished compared to controls as measured by RT-qPCR ($P<0.05$).

Conclusion. Our data suggests that WDR61 is involved in cyclic changes within the endometrium to prepare for blastocyst implantation during the WOI. When it is dysregulated in vivo, WDR61 may contribute to RIF due to the impaired adhesive capacity of the luminal epithelium. These findings could be translated to the clinic to enhance current IVF in women with implantation failure.

abs #107

Uncovering the vascular effects of insulin: the regulation of endothelin-1 in pregnancies complicated by gestational diabetes

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Background. Gestational diabetes mellitus is a condition of pregnancy that can result in maternal endothelial and vascular dysfunction. Endothelin-1 (ET-1) is a potent vasoconstrictor that contributes to endothelial dysfunction, however its regulation in gestational diabetes is unclear.

Methods/Results. Maternal plasma was obtained from patients with gestational diabetes (n=16), and gestation-matched controls at term (n=19); circulating ET-1 levels were assessed by ELISA. Human omental arteries were dissected from omental fat biopsies collected at caesarean section(n=28); mRNA expression of ET-1 and its receptors, ETA and ETB, in addition to vascular cell adhesion molecule-1 (VCAM1) and intercellular adhesion molecule-1 (ICAM1) were assessed by qPCR. Circulating ET-1 levels and mRNA expression of ET-1, ETA, ETB, VCAM1 and ICAM1 were not different in patients with gestational diabetes.

Using wire myography, we investigated ET-1 (10-11M-10-4M) constriction in omental arteries from patients with gestational diabetes, compared to gestation-matched controls (n=7). Gestational diabetic cases were stratified by clinical management; either diet (n=5) or insulin (n=6) treatment during pregnancy. Additionally, we interrogated the effect of insulin on healthy pregnant arteries; omental arteries from healthy pregnancies (n=7) were incubated with insulin (10mUmL) or control for 30 minutes and ET-1 constrictions were then measured. Arteries collected from patients complicated by gestational diabetes and treated with insulin, in addition to ex vivo short-term incubation of healthy arteries with insulin, reduced ET-1 constriction.

Conclusion. Here we demonstrate that insulin treatment during pregnancy reduces ET-1 vasoconstriction of the maternal vasculature. These data suggest that insulin may improve vascular function in gestational diabetes, however further investigation is needed.

abs #157

The prevalence and impact of Molar Incisor Hypomineralisation in children attending specialist paediatric dental services in Australia

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Background. Molar incisor hypomineralisation (MIH) is a qualitative defect of enamel characterised by demarcated opacities. Aesthetic and functional sequelae of MIH could manifest as reduced oral health related quality of life (OHRQoL). This study aims to investigate the presentation and severity of MIH , hypomineralised second primary molars (HSPM) and OHRQoL of children.

Methods/Results. This cross-sectional study recruited children aged 7 to 16-years-of-age attending specialist paediatric dental clinics in Melbourne, Australia. Clinical examination utilised the validated European Academy of Paediatric Dentistry (EAPD) and ICDAS indices to quantify the presence and severity of MIH and caries. OHRQoL data was collected via the Child Perception Questionnaire and Parent-Caregiver Perception Questionnaire. Sociodemographic information was obtained. Descriptive statistics were employed to analyse the data.

A total of 112 children, with a mean age of 10 years old were examined over 12 months at both private and public specialist paediatric dental clinics. A total of 65 (58%) of participants were male and 42% (n=47) were female. Sociodemographic status (SES) was skewed as 75.7% of participants had a healthcare card and lower SES. The prevalence of MIH was 29% (n=33) with severe MIH in 78.8% (n=26) of affected children. HSPM was present in 14 (12.6%) participants. Untreated dental caries was present in 54.3% (n=60) with cavitated lesions (ICDAS>2) present in 39.8% (n=44) of children. The most common impacts on OHRQoL as reported by both children and caregivers related to mouth sores, bad breath, pain and food impaction.

Conclusion. MIH affects children attending specialist paediatric dental clinics in Australia.

abs #167

Feasibility of Home Blood Pressure Monitoring in the Paediatric Outpatient Clinic Setting

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Background. Hypertension affects over 6% of Neurofibromatosis type 1 patients, double the general youth population. Given the long-term health risks posed by childhood hypertension, blood pressure (BP) monitoring is required. Telehealth is an increasingly desirable option for families but is hindered by the need for in-person BP measurement. This study aimed to assess the feasibility and comparability of home-based BP monitoring.

Methods/Results. Fifty-two children and adolescents (5.8-18.3 years) from the Neurofibromatosis clinic at the Royal Children's Hospital were included. BP was measured thrice manually and once via an automated device (Omron HEM-7121) in the clinic, and daily at home for three days. A Children's Anxiety Meter Scale was also administered daily in both settings. Post-experiment, a parental survey was conducted, and devices were returned via pre-paid post. Home vs. clinic manual and automatic systolic BPs were similar (2.3±8.3mmHg, p=0.06 and 1.2±11.0mmHg, p=0.47 respectively). Home diastolic BP, and anxiety scores showed no significant variation between settings. Home BP monitoring was easy, took approximately 10 minutes, and minimally interrupted family life. Families largely preferred home BP monitoring (73.3% "yes", 23.3% "not sure" and 3.3% "no"), with 90.4% returned their devices.

Conclusion. Home BP monitoring proved feasible and comparable to in-clinic measurements, with no associated anxiety. Telehealth with home BP monitoring is a viable, preferred alternative to in-person appointments, fostering greater equity of care and access to preventative services. However, device return rates need improvement for future programs.

abs #170

Comparison of efficacy, immunogenicity, and safety of Typhoid Conjugate Vaccine (TCV) in children: a systematic review and meta-analysis

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Background. Typhoid fever, caused by Salmonella typhi, contributes to 11 – 21 million annual cases and 128 – 161 thousand deaths globally. Several Typhoid Conjugate Vaccines (TCV) are under development have shown to be effective and safe among children. Review on TCV's efficacy, immunogenicity, and safety are needed to support evidences to introduce vaccine into immunization programs.

Methods/Results. The major databases MEDLINE, EMBASE, and CENTRAL databases were searched to 14 August 2023. The review includes the phase I, II and III randomized controlled trials (RCTs) that assessed the efficacy, immunogenicity, and safety of the TCV. The network meta-analysis used a Bayesian model and used the surface under the cumulative ranking to rank the comparisons between the vaccines.

Conclusion. The result of this network meta-analysis is important to provide evidences to introduce vaccine into immunization programs in many countries. TCV showed high efficacy and are immunogenic and safe among children.

abs #173

Why SPINT1 is dysregulated in placental insufficiency and what its role is in physiological placental development

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Background. Low circulating Serine Peptidase Inhibitor Type 1 (SPINT1) - critical to murine placentation (Szabo et al 2007, *Oncogene*) (Tanaka et al 2005, *Mol Cell Biol*) – is associated with fetal growth restriction (FGR) (Kaitu'u-Lino et al 2020, *Nat Commun*). But what leads to aberrant expression in human placental dysfunction isn't yet known, nor its function in physiological development of placenta.

Methods/Results. Immunohistochemistry for SPINT1 performed on placental sections from across gestation localised the protein to the cytotrophoblast, while human Trophoblast Stem Cells (hTSC) (Okae et al 2018, *Cell Stem Cell*) model first trimester placenta, in which SPINT1 was also in differentiated lineages; syncytiotrophoblast (SCT) and extravillous trophoblast (EVT).

To determine what regulates SPINT1 expression, cells cultured in 8% O_2 were subject to different treatment conditions to observe the effect on *Spint1* mRNA and SPINT1 protein expression and secretion, including: (1) hypoxia (1% O_2), which reduced *Spint1* mRNA transcripts by 40% ($p<0.01$) and protein secretion by 50% ($p<0.01$); (2) siRNA knockdown of transcription factors CDX2, GRHL2, HIF-2a, which did not alter *Spint1* mRNA, however cellular and secreted SPINT1 was reduced with siGRHL2 ($p<0.01$), suggesting post-transcriptional modifications; (3) inhibiting MMP-mediated secretion using broad spectrum MMP-inhibitor Batimastat, which reduced SPINT1 secretion by 28% (10uM Batimastat, $p<0.05$).

SPINT1's function was investigated by silencing SPINT1 in hTSCs using siRNA and observing the effect on critical cellular functions: (a) proliferation, using xCELLigence, was decreased; (b) differentiation into SCT and EVTs was altered; and (c) inhibition of downstream proteolytic activity (fluorogenic peptide substrate), although there was no significant change, potentially due to compensatory upregulation of SPINT2.

Conclusion. This elucidates some cellular mechanisms underlying the decreased SPINT1 observed in FGR, better understanding its role in placental pathophysiology.

Epidemiology and Population Health

abs #004

Women are leading the socioeconomic gradient transition in non-communicable diseases in China: longitudinal national data analysis between 1991 and 2020

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Background. Non-communicable diseases (NCDs) are a major contributor to health and economic burden. Analysis indicates a positive relationship between SES and NCDs in low-income countries, but the relationship becomes negative in high-income countries. This reversal of the relationship between SES and NCDs in different stages of economic development has been labelled the "reversal hypothesis". The “reversal hypothesis” also suggests that the burden of NCDs shifts from the rich to the poor as a country's economic development progresses. This study aims to examine the SES-NCD gradient over the past 30 years in China and further clarify the gender difference and to explore the possible contributors to the difference.

Methods/Results. Using nine waves of China Health and Nutrition Survey (1991-2015) and six waves of the China Family Panel Studies (2010-2020), we investigated SES-NCD gradient among participants aged 18 years and above. We used self-reported doctor diagnosed conditions to identify NCDs and used educational attainment as the proxy of SES. Multivariable logistic regression models were used to examine changes in SES-NCD gradient, and the Blinder-Oaxaca (BO) decomposition were used to explore the contributors to the difference.

The results shows that NCD prevalence increased from about 3% in 1991 to nearly 14% in 2020 in China. NCD prevalence was always lower among higher SES women compared to low SES women, whereas the prevalence was in general similar between high SES and low SES men. The regression results show that the relationship between SES and NCDs are shifting from positive to negative over the past 30 years, whereas an earlier reversal happened among women compared to men. The preliminary Blinder-Oaxaca decomposition results indicated that the difference of NCD prevalence is significant at the 5% level among high and low SES women, but not among high and low SES men in most survey years. Most of the differences in NCDs prevalence between high and low SES groups in men and that in women were attributed to the different distributions of explanatory variables included in the model. However, the most important contributing factors affecting the difference between high and low SES groups were different in women and men. Specifically, the differential distribution of age (positive), overweight (positive), employment status (positive), insurance (positive), region (positive), and residency (negative) had the greatest contribution to the disparity among low and high SES groups in women, whereas the differential distribution of age (positive), overweight (negative), drinking (negative), smoking (negative) and employment status (negative) had the greatest contribution to the disparity among low and high SES men.

Conclusion. The findings from this study suggest China has experienced a reversal in the SES gradient of NCDs and women is leading the transition. Compared with men, the factors that cause the difference NCDs within women are more complex and significant. Targeted policy response is required to reduce the burden of NCDs in the transition economy, especially among low SES women.

abs #008

Culturally Safe Dental Practice

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Background. This project aims build on pilot research undertaken in 2021 to explore the needs and perceptions of Aboriginal people to inform a culturally safe dental model of care. The aim of the study is to describe a culturally safe dental practice, understand how it can be evaluated, and identify the barriers and enablers to achieving this.

Methods/Results. The study will use interviews and Yarning circles to explore the perceptions and needs of Aboriginal people in 2 states, Victoria and Northern Territory, with 3-4 settings in each to inform a culturally safety model. Which will then be tested and refined using interviews with 8 key informants. Data collection is to start in 2024.

Conclusion. The findings will be returned to the communities and the health services for their use, and to educational and policy settings to enable adaptations to meet the needs of Aboriginal and Torres Strait Islander peoples.

abs #014

Applying machine learning to electronic medical records to phenotype people who inject drugs for hepatitis C surveillance

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Background. People who inject drugs are most at risk of hepatitis C infection and related sequelae. In Australia, routinely extracted de-identified electronic medical records (EMR) from sentinel clinics are used for hepatitis C surveillance, however these lack systematically collected behavioural risk factors making it challenging to identify and characterise (phenotype) risk groups. Machine learning techniques have been successfully applied to similar problems.

Methods/Results. We used Random Forest classification on EMR data from primary care clinics to develop a phenotyping model. A labelled sample was used to train and test the model (70:30 split) which included 88 predictor variables for 2422 people who inject drugs and 2422 randomly selected patients. Performance was measured using accuracy (correct predictions/total) and a manual review of false positive predictions was conducted. We iteratively trained and tested the model, each time: removing the most important predictors to uncover variables that contributed to phenotyping people who inject drugs; and adding reclassified people who inject drugs to improve model quality.

The model demonstrated high accuracy (92.9%). The most important predictors were opioid agonist treatment prescriptions and total hepatitis C tests proportional to time between first and last clinical visit. Less obvious predictors included general prescriptions, whether care was provided by a doctor or nurse, time between first and last clinical visit and HIV testing.

Conclusion. We built a highly predictive model and identified important risk factors for phenotyping people who inject drugs using EMRs. Further evaluation is needed to ensure it is accurate and scalable for national hepatitis C surveillance.

abs #016

Optimal digital mindfulness programs in improving mental health outcomes in young adults: a systematic review and meta-analysis

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Background. Mindfulness practice has garnered increasing interest for its potential to improve mental health. Traditionally taught face-to-face, over the past 40 years mindfulness programs have been translated into digital formats, and these formats have been shown to be equally effective for mental health management to in-person formats. Programs differ in course content, features, delivery mode, and format, all of which may affect the practice adherence required for improved wellbeing. It is unknown whether these technical and practice aspects impact health outcomes.

Methods/Results. Cochrane systematic review guidelines were applied. A literature search was performed in databases of EMBASE, Medline, CINAHL, Cochrane library, and PsycINFO. Studies were restricted to randomised controlled trials (RCTs) and quasi-RCTs, reporting on the efficacy of digital mindfulness programs on mental health outcomes in young adults aged 18-30 years. Only articles written in English were retained. Study quality was assessed using the Cochrane RoB 2 tool.

The search strategy extracted 1059 articles. Two independent reviewers conducted abstract and full-text screening based on eligibility criteria. Thirty-seven studies were included. The impact of digital mindfulness program aspects, including content, delivery mode, duration, frequency, and participant adherence to mindfulness practice, to improve stress, depression, and anxiety have been compared.

Conclusion. Results may inform components of digital mindfulness programs that impact optimal mental health outcomes, which may provide valuable insights for the design of future digital mindfulness programs.

abs #019

The role of economic evaluation in modelling non-pharmaceutical interventions for pandemic policy: a systematic review

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Background. Dynamic transmission modelling provides epidemiological guidance to pandemic policy making. It is not clear how economic modelling is incorporated to also generate cost-effectiveness estimates of pandemic policy responses.

Methods/Results. We systematically searched the databases Embase, Pubmed and Scopus for relevant studies, with no date restriction, on 23 November 2022.

Selection criteria: Dynamic modelling studies that incorporated economic evaluation of NPIs for infectious diseases, including Ebola, Zika, Influenza H1N1, Influenza H5N1, MERS, SARS and COVID-19.

Data collection and analysis: Recovered citations were screened by two independent reviewers, and eligible studies were included for extraction of study features. Included studies were then scored on their utility for policymakers using criteria developed by the authors.

Results: Of 1,804 screened studies, 40 met the selection criteria. 31 (78%) studies modelled SARS-CoV-2. A range of NPIs were considered, including school closure, testing/screening, lockdown, isolation or quarantine, social distancing and mask use. Most studies, 21 (53%), utilised an extension of a Susceptible-Exposed-Infectious-Recovered (SEIR) compartmental model. The most common type of economic evaluation used was cost-effectiveness analysis, used in 20 studies (50%), followed by cost-utility analysis used in 12 studies (30%), and cost-benefit analysis used in 14 studies (35%).

Conclusion. Economic evaluation has been seldom incorporated in dynamic modelling of NPIs. Although there has been an increase in these modelling studies since the COVID-19 pandemic, there remain large gaps in this literature. While the literature is sparse, integrated economic-epidemiological modelling has the potential to assist decision making in the future, both in planning for and during pandemics.

abs #027

Subsequent Primary Cancer Risks for Colorectal Cancer Survivors: A Systematic Review and Meta-Analysis

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Background. Cancer can occur more than once in a person's lifetime, and for colorectal cancer survivors, the risk of developing subsequent primary cancer (SPC) in the colorectum and other sites is a significant concern. This systematic review aimed to estimate the risks of SPC for colorectal cancer survivors

Methods/Results. We screened peer-reviewed articles published before September 2022 through four databases to identify studies that used population cancer registry data to estimate the standardised incidence ratios (SIRs) of SPC for colorectal cancer survivors. We summarised the reported SIRs and then calculated age-specific cumulative risks of SPCs for colorectal cancer survivors from different regions: North America, East Asia, Europe, and Australasia using the summary SIRs and age-, sex-, region-, calendar- and cancer-specific 5-year incidences for the general population.

Of 7403 articles identified, we included 47 in the meta-analysis. The risks of any SPC, extracolonic SPC and subsequent colorectal cancer were higher than the risk for first primary cancer for the general population (pooled SIR 1.11, 95% Confidence Interval (CI) 1.04–1.19; SIR 1.18, 95%CI 1.12–1.25; and SIR 1.47 95%CI 1.23–1.70, respectively). For specific organs, we found evidence of increase risks of SPC in the small intestine, testes, ovary, uterus, kidney, Thyroid, and female breast. The estimated cumulative risks of any SPC, extracolonic SPC, and subsequent colorectal cancer to age 75 years for both sexes combined were: 43.1%, 41.3%, and 10.5% in Australasia; 39.4%, 38.9%, and 8.22% in North America; 39.2%, 36.7%, and 11.5% in East Asia; and 37.2%, 35.4%, and 5.96% in Europe, respectively.

Conclusion. The findings indicate that individuals who have survived colorectal cancer face a greater likelihood of developing SPC in both primary and extracolonic sites compared with those who have not had colorectal cancer. The estimated cumulative risks could be useful in guiding medical professionals to determine the most effective surveillance approaches for colorectal cancer survivors.

abs #061

Exploring the population dynamics and virulence determinants of Salmonella Virchow prevalent in Victoria, Australia

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Background. Australia has one of the highest burdens of acute gastroenteritis among high income countries. The causative agent is non-typhoidal Salmonella (NTS), comprised of over 2,500 serovars. In certain cases, NTS infections might lead to the more serious blood-stream infections, caused by invasive non-typhoidal Salmonella (iNTS). In Australia, Salmonella enterica serovar Virchow has been identified as an iNTS serovar. To date, the genomic epidemiology and underlying genetic mechanisms mediating the ability of S. Virchow to cause disease in humans, is poorly understood.

Methods/Results. We undertook whole genome sequencing using Illumina platforms of 413 S. Virchow isolates collected from human patients in Victoria, Australia, over a 20-year span. We sought to study their population structure, antimicrobial resistance (AMR) and genome content. We further selected representative isolates for long read sequencing using Oxford Nanopore Technologies (ONT), to assemble complete reference genomes.

We identified 4 sequence type (ST) dependent lineages of S. Virchow from the inferred phylogeny. STs are determined based on allelic variations in the housekeeping genes of isolates. These lineages differ in the genome content of Salmonella pathogenicity islands and potential virulence proteins. Limited AMR mechanisms were detected in genome screens, suggesting that most S. Virchow isolates are susceptible to recommended therapeutic options.

Conclusion. We conclude that multiple lineages of S. Virchow have been circulating in Australia, with potential differences in virulence properties. Future analyses will focus on understanding the infection patterns of different S. Virchow STs and characterise the virulence determinants through genomics and laboratory-based studies.

abs #074

Disparities in survival after diagnosis of cervical cancer between tribal and rural women from Tamil Nadu, south India

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Background. Given the scarcity of epidemiological data on cervical cancer in tribal (Indigenous) subgroups in India, this analysis compared the survival experience of women with cervical cancer from tribal and rural Tamil Nadu.

Methods/Results. A non-concurrent population based cohort study was carried out by following up women diagnosed with cervical cancer between 2013 and 2022, from a rural area (total population 116,000), and a hilly tribal area (population 40,000), based on a patient list created from admissions to a tertiary center, primary care services, as well as deaths identified in the area.

Survival status was obtained from death registers and follow up by community health workers. Analysis was by comparing Kaplan Meier curves using the log rank test, and Cox regression.

Of 29 tribal and 43 rural women, 54% and 45% respectively were diagnosed in later stages (3 or 4), (chi-square p 0.474.) Five year survival was 32% in tribal women and 54% in rural. Median survival after diagnosis was 14 months (95% CI: 0-31) for tribal women, compared to 27 months (95% CI: 11 – 43) for rural women, log rank test p value 0.074 for Kaplan Meier curves. Tribal women had 2.16 times higher (95% CI: 1.05– 4.44) five year mortality, after adjusting for age and stage.

Conclusion. Tribal women with cervical cancer had twice the mortality of rural women, even after adjusting for age and stage at diagnosis. This inequity could potentially be reduced by facilitating culturally appropriate screening and follow up services supporting treatment completion and ongoing care after diagnosis.

abs #081

Multimorbidity- The burden and impacts of Multimorbidity in a Hospital in India

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Background. Multimorbidity- the presence of more than two chronic diseases in an individual- is growing in prevalence and is a major contributor to polypharmacy, increased mortality, and poor quality of life (QoL). In India, the community prevalence of multimorbidity is 28.3% and most patients receive healthcare in hospitals. This study aimed to determine the prevalence of multimorbidity and associated patient factors in Christian Medical College Vellore, a tertiary care hospital., and an affiliated associated secondary care hospital, in India.

Methods/Results. Patients attending outpatient clinics at tertiary, secondary care and inpatient care at CMC Vellore in South India were recruited between 2020 and 2022. Patients were assisted to complete a self-reporting questionnaire regarding their health and its associated impacts.

42.11 % of patients had multimorbidity, with diabetes mellitus (79.2%) and hypertension (76.5%) being the most common conditions. Most patients were aged <60 years (57.4%). Polypharmacy was common (36.4%), 73.9% had drug interactions and 21.6 % reported that they couldn't afford their medications. The mean physical and mental scores The quality of life scores on the SF12 (QoL scale) were 40.5(SD-6.4) for the physical component.19.6% of inpatients died during their admission.

Conclusion. Multimorbidity in our hospital service in Southern India is common and is associated with suboptimal physical and mental health. Polypharmacy is common but further research is needed to understand the cost burden and impact on medication adherence.

These results will be used to inform the design of an intervention to coordinate care for people with multimorbidity in our hospital.

abs #082

Six ways the phenomenon of coerced "self-produced" child sexual exploitation material occurs: A Critical Interpretive Synthesis of the Literature

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Background. There is an emerging global issue of children and young people being coerced to 'self-produce' sexual images and videos of themselves and upload this content on the internet, these images and videos are referred to as 'child sexual exploitation material' (CSEM). Last year the International Watch Foundation confirmed that 'self-produced' CSEM represented majority of CSEM webpages reported over a 12-month period.

Methods/Results. This review explored how the phenomenon of coerced 'self-produced' child sexual exploitation material has been constructed in the literature using Critical Interpretative Synthesis. Relevant keywords were systematically searched on the data bases ASSIA, OVID, CINAHL, ERIC, Family & Society Studies Worldwide and Google Scholar. Inclusion criteria included: Peer reviewed research articles, conceptual papers, commentary papers, dissertations, thesis, book chapters, systematic reviews, and Government Reports, peer reviewed journal articles; focus on children who self-produce child exploitation material; and published in English between January 2005 and November 2022. The initial search identified 1,020 papers, after duplications were removed two reviewers read the title and abstract of the remaining 957 papers. 108 papers met inclusion criteria and two reviewers completed a full text review. 37 papers were included in the final sample. The findings are described and critically analysed through a perpetrator accountability lens. Findings indicate there are six models of 'self-produced' child sexual exploitation material: Solicitation; Sextortion; Viral Challenge; Economic; Peer Sexting; and Social Media.

Conclusion. These models can provide a conceptual framework to help practitioners further understand the complex nuances that exist within the phenomena of coerced 'self-produce' CSEM whilst holding perpetrators accountable for their abusive actions.

abs #088

"Everyone learns differently based on their interests and field": doctors' experiences of learning genomics in the workplace

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Background. Advances in genomic technologies are encouraging large-scale efforts to implement genomics into routine healthcare internationally. Such efforts are creating more genomics workplace learning opportunities, which may help address the lack of a genomic-competent health workforce. However, there is a lack of genomics workplace learning research in the daily work context. We aimed to explore doctors' current genomic workplace learning and identify factors influencing their experiences and perspectives.

Methods/Results. Doctors working in hospitals with access to Genetics resources in Victoria, Australia were invited to participate in this longitudinal phenomenological study by completing an online screening form. Those meeting eligibility criteria submitted six reflective narratives over a six-month period, with semi-structured interviews before and after. Reflexive thematic analysis was used to deductively and inductively identify codes and themes. Ten participants were recruited, representing a range of specialties, career stages, and genomics experiences. An array of genomic workplace learning experiences stemmed from participants' routine work and engagement. Delivering best patient care and navigating healthcare systems were reported as rich learning experiences. Participant experiences and perspectives were influenced by a unique combination of factors, including their specialty, workplace, access to funded genomic tests, and interactions and relationships with genomics-knowledgeable colleagues.

Conclusion. While workplace learning is currently under-recognised in building the workforce's capacity to practice genomics, these findings reveal the relevant and impactful experiences afforded by everyday work contexts. Our findings provide evidence to consider workplace learning as complementing structured education initiatives to help prepare the workforce for genomics implementation in routine healthcare.

abs #089

What is the relationship between women's economic empowerment and basic water, sanitation, and hygiene in the home?

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Background. The world is not on track to achieve universal and equitable access to safe water, sanitation, and hygiene (WASH) by 2030 under SDG 6. Lack of adequate WASH services has a disproportionate effect on women. We aimed to investigate the relationship between women's economic empowerment and basic WASH in the household.

Methods/Results. We analyzed a sample of 278,536 married women in 31 low- and middle-income countries using DHS survey data. We created an economic empowerment score based on having a bank account, a mobile used for financial transactions and deciding about household purchases and how to spend her and her partner's money. We then investigated its association with basic WASH in the household. More empowered women were significantly more likely to live in a household with basic WASH in urban and rural settings. The most empowered women had a basic water prevalence 18.3 percentage points (pp) higher than the least empowered. For basic sanitation, it was 26.9 pp higher and 32.7 pp for basic hygiene. There were large inequalities both within and between countries. Multiple countries had inequalities in WASH prevalence between the most and the least empowered women higher than 40 percentage points.

Conclusion. Our study adds to the growing body of evidence of women's empowerment as a pathway to better WASH and vice-versa, using a nationally representative, multicountry sample. Cash transfer programs aiming to improve WASH infrastructure could explore women's economic empowerment both as a goal and as a possible tool for higher effectiveness.

abs #096

Overlapping transmission of group A and C/G Streptococcus facilitates inter-species mobile genetic element exchange

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Background. Streptococcus dysgalactiae subspecies equisimilis (SDSE) and Streptococcus pyogenes share skin and throat niches with extensive genomic homology and horizontal gene transfer (HGT) possibly underlying shared disease phenotypes. It is unknown if cross-species transmission interaction occurs. We conducted a genomic analysis of a longitudinal household survey in remote Australian First Nations communities for patterns of cross-species transmission interaction and HGT.

Methods/Results. SDSE and S. pyogenes isolates from throat and skin sore swabs were re-analysed from a monthly surveillance study conducted between 2003 to 2005. From 4,547 person-consultations across two communities, 294 SDSE and 315 S. pyogenes isolates were whole genome sequenced. Transmission networks were inferred from genomic and epidemiological metadata. SDSE and S. pyogenes transmission intersected extensively among households and the observed co-occurrence and transmission links were consistent with independent transmission without inter-species interference. At least one of three near-identical cross-species mobile genetic elements (MGEs) carrying antimicrobial resistance or streptodornase virulence genes was found in 55 (19%) SDSE and 23 (7%) S. pyogenes isolates.

Conclusion. SDSE and S. pyogenes demonstrate extensive co-circulation with evidence of shared MGEs carrying biologically important virulence and antimicrobial resistance genes. These findings support a need to integrate SDSE and S. pyogenes surveillance and control efforts.

abs #101

Population structure, serotype distribution and resistance mechanisms of third-generation cephalosporin-resistant E. coli in Australia

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Background. In Australia, Escherichia coli is a major cause of hospital and community-acquired extra intestinal infections. The increasing prevalence of resistance to third-generation cephalosporins in E. coli represents a global public health concern due to high rates of morbidity and mortality. Further, increased treatment costs and times against these drug resistant E. coli is a significant economic burden. However, information on population structure, serotype distribution and resistance mechanisms of third-generation cephalosporin-resistant (3GC-R) E. coli remains limited in Melbourne, Victoria.

Methods/Results. To address this, 929 3GC-R E. coli isolates were collected from extra-intestinal sites across four Melbourne hospital networks and underwent whole genome sequencing. Core genome analysis, in silico MLST, phylogrouping and serotyping were performed to determine the population structure and serotype distribution. abriTAMR with AMRFinderPlus database was used to detect resistance genes. These analyses revealed a highly diverse E. coli population with 98 unique STs detected, 10 STs with >7 isolates (n= 774/929, 83%) were found in >2 hospitals. The most abundant sequence type was ST131 (n=460/929, 49%), with 83% of the ST131 typed as serotype O25:H4. The pre-dominant 3GC-R gene was blaCTX-M-15 (n=386/929, 41.5%) and found in 55 STs. Multidrug resistance (MDR) (genotypically resistant to □3 antibiotic classes) was also identified in 67% isolates across 98 different sequence types.

Conclusion. Our study revealed high genomic diversity among 3GC-R E. coli in Melbourne hospitals with significant prevalence of MDR isolates, particularly globally recognized and challenging ST131 carrying blaCTX-M-15 gene. The ongoing circulation of different MDR STs in hospitals requires further investigation into the transmission dynamics.

abs #104

Birthing on Instagram: exploring the portrayal and representation of caesarean section among Indonesians

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Background. Caesarean section (CS) rates in Indonesia are rapidly increasing, and there is an urgent need to understand what social factors are driving these increases. Social media is also increasingly popular, providing a platform for users to seek and exchange information, including health-related information. As social media can be a unique platform to explore how it may influence women's preferences towards CS, this study explored how CS is portrayed and represented by Indonesians.

Methods/Results. We downloaded Instagram posts using CS #hashtags and extracted post's attributes (image, text, #hashtags). We used a mixed-methods approach to analysis, utilising natural language processing, quantitative, and qualitative content analysis. A total of 9,978 posts were analysed quantitatively and 720 randomly sampled posts were analysed qualitatively.

We found unbalance information shared about CS. Instagram posts using CS #hashtags were mostly advertising natural medicine products to women for faster CS recovery. Services were advertised to offer women a clinical consultation to choose an auspicious day for childbirth – encouraging uptake for elective CS. Some health facilities and providers explicitly promoted CS by giving discounts for the procedure, and promoting advanced clinical techniques to improve comfort, painless birth and faster recovery. Stigma towards women who underwent CS was common, highlighting structural sexism towards women.

Conclusion. This study is a call for Indonesia to begin efforts in regulating advertisements around services and medical procedures during birth by health facilities and providers, monitoring CS at the health facility level, and ensuring that CS is delivered to women who medically need it.

abs #105

Genomic insights into the longitudinal transmission of Neisseria gonorrhoeae in Australia

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Background. The ongoing transmission of Neisseria gonorrhoeae, the cause of gonorrhoea, is a significant public health challenge. Despite improved access to prevention and treatment, Australia has seen a resurgence in gonorrhoea cases, with 33,692 reported in 2022, a 105.7% increase since 2012.

Methods/Results. We investigated the genetic diversity and antimicrobial resistance of N. gonorrhoeae isolates in Victoria from January 2017 to July 2021, including the COVID-19 period. Using cgMLST and Bayesian methods, we grouped 5,881 isolates into 233 genomic clusters, with 38.7% (12/31) of large clusters (≥30 genomes) showing persistent transmission for over 24 months. Persistence was significantly associated with larger clusters (OR 1.02, p=0.028) and clusters with a higher proportion of women (OR 2.91, P = 0.017). For the front-line dual treatments, phenotypic susceptibility to azithromycin was significantly associated with persistence (OR 127, $p < 0.0001$), while phenotypic resistance to ceftriaxone showed a near significant association with persistence (7.7, $p = 0.057$). Following COVID-19 public health restrictions, we observed a decrease in transmission, leading to a decrease in genomic diversity, including a decrease in diversity of antimicrobial resistance determinants, although no significant changes in phenotypic resistance.

Conclusion. The whole genome sequencing of N. gonorrhoeae isolates collected in Victoria has provided valuable insights into the longitudinal transmission patterns and genetic diversity of the pathogen. Phenotypic resistance to frontline treatment options reveals significant associations with cluster persistence, underscoring the importance of ongoing monitoring of antimicrobial resistance and transmission dynamics of N. gonorrhoeae to inform effective control strategies and optimise treatment guidelines.

abs #106

Diet-wide association study for the incidence of type 2 diabetes mellitus in community-dwelling adults using the UK Biobank data

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Background. This prospective cohort study aims to investigate the relationship between a vast spectrum of dietary food and nutrient intakes and the onset of type 2 diabetes mellitus (T2DM) by using Diet-wide association studies (DWAS).

Methods/Results. We included 119,040 UK Biobank participants without T2DM at the baseline, with a median follow-up of 11.7 years. The DWAS analysis was utilised Cox regression models, to analyse the pseudo-numerical associations between the quintiles of dietary intake factors and the T2DM incidence. Bonferroni correction method was applied to control false positives. Manhattan plots and forest plots were introduced for result visualisation.

A total of 23 out of 224 dietary factors were found to be significantly associated with the T2DM risk. Among these, white wine (Hazard ratio [HR] = 0.90, 95% confidence interval: 0.83-0.98, p-value [p] = 0.012), red wine (HR = 0.90, 0.88-0.93, p = 0.006), and fresh tomatoes (HR = 0.90, 0.88-0.93, p = 2.50 x10^(-15)) showed a negative association with T2DM risk. Conversely, sliced buttered bread (HR = 1.08, 1.06-1.11, p = 4.55 x 10^(-10)) exhibited a positive association. For dietary nutrients, 7 out of 21 nutrient intake revealed significant associations. Starch (HR = 1.03, 1-1.06, p = 0.0218) is the sole nutrient risk factor while the rest of the nutrients are protective for T2DM.

Conclusion. DWAS can be considered an effective method for discovering novel associations when exploring a vast number of dietary variables simultaneously and may provide valuable insight into future dietary guidance instruction for T2DM.

abs #132

The prevalence of six perinatal anxiety disorders in low- and middle-income countries: a systematic review and meta-analysis

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Background. Anxiety disorders are associated with poor maternal and neonatal outcomes. Their prevalence in low- and middle-income countries (LMIC) is unclear. We set out to determine the prevalence of six anxiety disorders among pregnant and postpartum women in LMICs.

Methods/Results. We conducted a systematic review and meta-analysis of studies reporting the prevalence of generalised anxiety disorder, obsessive-compulsive disorder, social anxiety disorder, post-traumatic stress disorder, panic disorder or adjustment disorder during the perinatal period (conception to 12 months postpartum), using a validated method. We only included studies from LMICs. Random effects meta-analysis and meta-regression were used to estimate pooled point prevalence.

We identified 8,106 studies. 115 met the inclusion criteria, reporting outcomes of 118,785 women from 24 LMICs. Generalised anxiety disorder was most commonly reported (99 studies) and was the most prevalent at 20·1% (95% CI 17·0 – 23·5; n=104,475). Post-traumatic stress disorder was the second most prevalent, affecting 9·6% (95% CI 5·9 – 14·3; 30 studies; n=31,338) of women. Panic disorder was the least prevalent at 2·1% (95% CI 0·9 – 3·8; 6 studies; n=4,641). The prevalence of generalised anxiety varied by country income status, with the highest prevalence among lower middle-income countries (27·0% [95% CI 19·0 – 35·2]; 34 studies; n=12,941), followed by upper-middle-income (17·6% [95% CI 14·5 – 21·1]; 59 studies; n=90,932) and low-income countries (range 1·0% – 28·2%; 3 studies; n=1,052).

Conclusion. In this large systematic review, we found one in five women in LMICs experience anxiety disorders during pregnancy and postpartum. Targeted action is needed to reduce this alarmingly high burden of anxiety disorders.

abs #137

High performance enrichment-based genome sequencing to support the investigation of hepatitis A virus outbreaks

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Background. Hepatitis A virus (HAV) infections are an increasing public health concern in low-endemicity regions due to outbreaks from foodborne infections and sustained transmission among vulnerable groups, including persons experiencing homelessness, those who inject drugs, and men who have sex with men (MSM), which is further compounded by aging, unvaccinated populations. DNA sequence characterisation of HAV for source tracking is performed by comparing small subgenomic regions of the virus. While this approach has been successful when robust epidemiological data are available, poor genetic resolution can lead to conflation of outbreaks with sporadic cases. HAV outbreak investigations would greatly benefit from the additional phylogenetic resolution obtained by whole virus genome sequence comparisons. However, HAV genomic approaches can be difficult because of challenges in isolating the virus, low sensitivity of direct metagenomic sequencing in complex sample matrices like various foods such as fruits, vegetables and molluscs, and difficulty designing highly multiplexed PCR primers across diverse HAV genotypes.

Methods/Results. Here, we introduce a proof-of-concept pan-HAV oligonucleotide hybrid capture enrichment assay from serum and frozen berry specimens that yields complete and near-complete HAV genomes from as few as four input HAV genome copies. We used this method to recover HAV genomes from human serum specimens with high C_T values (34·7—42·7), with high assay performance for all six human HAV sub-genotypes, both contemporary and historical.

Conclusion. Our approach provides a highly sensitive and streamlined workflow for HAV WGS from diverse sample types, that can be the basis for harmonised and high-resolution molecular epidemiology during HAV outbreak surveillance.

abs #146

Web-Based STI/HIV Testing Services in Australia: Systematic Review and Analysis

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Background. Sexually transmitted infection (STI) rates continue to rise in Australia and timely access to testing and treatment is crucial to reduce transmission. Web-based services have been viewed as a way to improve access to STI/HIV testing and have proliferated in recent years. However, the regulation of these services in Australia is minimal. This review was systematically identified and assess web-based HIV/STI testing services available in Australia.

Methods/Results. A Google search of Australian web-based STI testing services was conducted March 2022 and repeated September 2022 using Boolean operators. The first 10 web-pages were assessed, and services categorized as self-testing, self-sampling or self-navigated pathology. Service reliability was assessed against the Health-on-the-Net Foundation Codee, and quality assessed using a score card we developed based on Australian guidelines. Additional factors were assessed including cost, time till results and rural access.

Seventeen services were identified (8 self-testing, 2 self-sampling, 7 self-navigated pathology). Only 4 offered recommended testing for all four infections (chlamydia, gonorrhoea, syphilis, HIV) at appropriate genital, anorectal and oropharyngeal sites. 5 services offered tests not recommended by guidelines (e.g. ureaplasma). Reliability scores (scale 0-8) were similar between all services (range 4.75-6.0, p=0.115). Quality scores (scale 0-58) were similar between self-navigated pathology and self-sampling services (average 44.89 and 44.75 respectively) but lower for self-testing services (22.66, p=0.002). Cost for services varied between self-navigated pathology ($0-595), self-sample ($0) and self-testing ($0-135).

Conclusion. There was considerable variability in quality and reliability of services identified. It is imperative that Australia develops standards to ensure the standard-of-care offered by web-based STI/HIV testing services are appropriate.

abs #147

Preferences for an online STI testing service; reaching and garnering trust with young people in underserved populations- a survey of young Australians

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Background. Sexually transmitted infection (STI) rates continue to rise but in-person testing services are unable to meet these demands resulting in the proliferation of online testing services. This study aims to explore the preferences of young people from diverse backgrounds on what they prefer from an online STI testing service.

Methods/Results. Australians aged 16-29 were recruited via digital media completed an online survey. Logistic regression was used to determine preference for online vs in-person services. Descriptive statistics was used to describe preferences of young people regarding trustworthiness, language used by the website, and how it's advertised.

905 people responded to the survey. 46% were cis-gendered heterosexuals, 20% MSM, 24% were CALD with 15% of Asian descent. 21% lived in rural areas. 75% preferred online testing over in-person testing; nearly twice as likely to live in rural areas (OR=1.90, p=.0053). CALD populations (OR=0.58, p=.0042) and those of Asian descent (OR=0.50, 0.0019) were less likely to prefer online testing.

Professionally supported services (98%) and privacy/confidentiality (89%) were selected by most participants as trustworthy services. How to advertise an online STI testing service varied among the populations with new media (e.g. social media) (84%) and collateral materials (eg. posters) (79%) being the top preferences by most people. Participants from rural areas (87%) and of Asian descent (88%) preferred posters as a means of advertising services.

Conclusion. These results indicate that there is an overall preference for online STI testing services but varied among different populations.

abs #162

Antibiotic resistance in Mycoplasma genitalium: temporal changes in prevalence and regional differences

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Background. Our previous meta-analysis of data to the end of 2017 found global macrolide, fluoroquinolone, and dual-class resistance in Mycoplasma genitalium had reached 51.4%, 9.3%, and 4.0%, respectively; prevalence was highest in the Western-Pacific. We updated this review to estimate current global antimicrobial resistance prevalence in M.genitalium.

Methods/Results. PubMed, Embase, and MEDLINE were searched for studies reporting macrolide and fluoroquinolone resistance-associated mutations in M.genitalium-positive samples published between 7 January 2019 and 18 April 2023, the period since our original meta-analysis. Data were extracted by year of sample collection (before 2012, 2012–2014, 2015–2017, 2018–2021) and geographic region. Pooled prevalence estimates (95% confidence intervals [CI]) were calculated using random-effects models.

Overall, 166 studies (59 from the previous review and 107 new studies from this update), were eligible for inclusion. We found that global macrolide resistance had decreased from 42.0% (95% CI: 35.6–48.6%) in 2015–2017 to 32.5% (26.5–38.8%) in 2018–2021, although in Europe, prevalence continued to increase. Global fluoroquinolone resistance remained stable at around 13.9% and prevalence was highest in the Western-Pacific (38.0%; 26.1–50.6%). Global dual-class resistance increased slightly from 5.2% (2.7–8.1%) in 2015–2017 to 6.9% (3.5–11.2%) in 2018–2021, also highest in the Western-Pacific (36%; 10.6–66.4%).

Conclusion. Macrolide resistance appears to be decreasing globally. Plausible explanations include reduced use of single-dose azithromycin as first-line therapy and the uptake of resistance-guided therapy for M.genitalium. Fluoroquinolone and dual-class resistance remain low but their slow increase is concerning as limited antibiotics remain for treatment.

abs #172

E. coli contamination of drinking water sources in rural and urban settings of 38 low- and middle-income countries

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Background. The world is not on track to achieve universal access to safely managed water services by 2030, and access is substantially lower in rural areas. This target, and many other global indicators, rely on the current classification of improved water sources for monitoring access. We aimed to investigate contamination in drinking water, comparing improved and unimproved sources in urban and rural areas.

Methods/Results. We analyzed a sample 95,070 households in 38 low- and middle-income countries, using MICS survey data. Samples from the household's water source and from a glass of drinking water were tested for E. coli contamination. We calculated the contamination prevalence according to source and area of residence. Contamination was widespread and unacceptably high in almost all countries, settings, and sources, with substantial inequalities between and within countries. Water contamination was found in 51.7% of households at the source and 70.8% at the glass of water. Some improved sources were as likely to be contaminated as unimproved sources, with contamination at the source found for 84.7% of households using rainwater and 89.0% using protected wells. Some sources, like piped water, were considerably more likely to be contaminated in rural than urban areas (contamination prevalence at the source of 49.3% vs 19.9%, respectively), while no difference was observed for other sources.

Conclusion. Monitoring water contamination, along with further investigation in water collection, storage, and source classification, is essential and must be expanded to achieve universal access to safe and clean water.

Infection & Immunity

abs #007

Exploring the molecular signatures of Staphylococcus aureus transition into human serum using multi-omics analysis

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Background. Staphylococcus aureus is a common human commensal bacterium. However, these colonising bacteria can invade the bloodstream, causing devastating infections, aggravated by the widespread emergence of resistance to last-line antibiotics like vancomycin. Understanding how S. aureus infection occurs has become increasingly challenging.

Methods/Results. In this study, we integrated multi-omics data including transcriptomics, proteomics, and metabolomics to identify the molecular signatures of S. aureus transition into human serum. We focus on five sepsis isolates representing the most common lineages of S. aureus that are clinically important in Australia. Our preliminary results show that distinct S. aureus isolates share common and strain-specific responses to human serum. Interestingly, the up-regulation of genes involved in iron acquisition pathways was conserved across S. aureus lineages. We observed that staphylobilin-forming heme oxygenase (isdI) was up-regulated, at both transcriptional and translational levels, in S. aureus exposed to human serum. Consistent with our analyses, an isogenic isdI mutant showed growth defects in human serum when compared to S. aureus wild-type JE2, suggesting the importance of iron uptake for bacterial survival in human serum.

Conclusion. For future analyses, multivariate data integration will be used to identify new potential conserved and strain-specific targets for further therapeutic treatments of sepsis.

abs #020

The polyphenol rich sugarcane extract (PRSE) has potential antiviral activity against influenza A virus in vitro

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Background. Influenza A virus (IAV) is one of the major global public health concerns. IAV is highly contagious and known for its high mutation rate which can generate pandemic strains with the potential for high mortality and morbidity. Therefore, IAV is now under annual surveillance at the global level. In addition to vaccination, antivirals are an option to combat IAV infection, and neuraminidase inhibitors have been the mostly used antivirals to treat IAV infection. However, the emerging resistance of IAV against neuraminidase inhibitors highlights the need for other potential alternative antivirals directed against IAV. Polyohenol rich sugarcane extract (PRSE) is an extract prepared from the molasses of a sugarcane plant that has anti-inflammatory, antioxidant and bactericidal activity. Thus, we aimed to evaluate it could also be used as an antiviral against IAV infection.

Methods/Results. Treatment of IAV-infected MDCK cells resulted in a dose-dependent virus inhibition when assessed by plaque assay and western blot. We could show that PRSE affected the early stages of viral replication including viral genome and mRNA transcription and viral protein expression. However, PRSE treatment of intact virions did not affect virus morphology, as assessed by electron microscopy, nor the ability of the virus to bind red blood cells. We extended our findings to show that PRSE had antiviral activity against a broad range of H3N2 and H1N1 IAV strains.

Conclusion. Overall, our findings show that PRSE has the potential to be used as an antiviral against IAV in vitro.

abs #029

Re-sensitizing Streptococcus pneumoniae to tetracycline and azithromycin antibiotics with metal ionophores

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Background. Streptococcus pneumoniae (the pneumococcus) is a globally significant bacterial pathogen and the leading cause of pneumonia mortality worldwide. Antibiotics remain critical in the treatment of active pneumococcal disease. However, multidrug resistant (MDR) isolates compromise our ability to effectively treat infections, necessitating alternative therapeutic approaches.

Previous studies have shown that metal intoxication of bacteria can increase their susceptibility to current antibiotics. Here, we use ionophores, a class of compound that facilitates unregulated shuttling of metal ions across the bacterial cell membrane, to rescue the efficacy of frontline antibiotics, tetracycline (TET) and azithromycin (AZI), against MDR S. pneumoniae isolates.

Methods/Results. Using minimum inhibitory concentration (MIC) assays, we show restoration of clinical sensitivity i.e., breakage of resistance, to AZI and TET in MDR S. pneumoniae clinical isolates upon co-administration with ionophores. At these ionophore-antibiotic concentrations, rapid bactericidal killing (≥3-log reduction in CFU compared to controls) was achieved within 4 hours of treatment.

Further analysis revealed significant hyper-accumulation of the metal ion under ionophore treatment which was potentiated when combined with TET or AZI. This corresponded with the upregulation of the metal-specific membrane efflux pump, indicating metal intoxication. Scanning electron microscopy (SEM) was conducted to assess the cellular morphology and membrane integrity, revealing visible disruptions to the cell membrane and cellular aggregation under individual and combined antibiotic-ionophore treatment.

Conclusion. This promising approach can rapidly provide alternate therapies and require less time, cost, and risk than traditional antibiotic development. Helping us to combat the alarming rise in MDR isolates and reduce the global burden of pneumococcal disease.

abs #040

The role of prophages and comGC in Streptococcus pneumoniae carriage duration

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Background. Streptococcus pneumoniae (the pneumococcus) is a human pathogen that colonises the nasopharynx (carriage). The duration of a carriage episode is associated with invasive potential and genetic recombination. However, very little is known about the factors influencing this process. Although some studies suggest a link between prophage sequences and shorter carriage duration, no experimental investigation has been conducted. Prophages tend to insert into specific sites in the bacterial chromosome, like the comGC gene (which encodes a major protein of Type IV pilus important for DNA uptake in pneumococcus) however little is known about the prevalence of this disruption by prophages. This study examined the role of prophages and comGC in carriage duration.

Methods/Results. To determine the effect of prophages on carriage duration, a phage mutant was created using PMP1335 background, designated ∆orf40[PMP1335]. When tested ∆orf40[PMP1335] in the infant mice carriage duration model, no difference in carriage duration or density was observed. To investigate the prevalence of prophage mediate disruption in the comGC gene, we screened a dataset of pneumococcal genomes from Asia. From the 258 genomes examined, 44 (17.1%) had the comGC gene disrupted by a prophage. To investigate the individual role of comGC in carriage duration a mutant strain from EF3030 background (serotype 19F) was created and is currently being tested in infant mice carriage duration model.

Conclusion. The presence of prophages has no effect on carriage duration. Current experiments are underway to determine if the loss of comGC (rather than the presence of phage) is the cause of the shortened duration.

abs #044

Improving the Proteomics Profiling of Intracellular Burkholderia cenocepacia K56-2 in THP-1 Macrophage Cells

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Background. Burkholderia cenocepacia is a bacterial pathogen that commonly infects individuals with cystic fibrosis (CF) where it replicates within lung macrophages yet our understanding of how B. cenocepacia subverts host macrophages is limited. Conventional proteomics profiling of infected macrophages is challenging due to the low infection index of B. cenocepacia, resulting in models characterised by predominately uninfected cells, which limits our ability to understand the host-pathogen interactions at play during infection.

Methods/Results. Using opsonization with polyclonal anti-B. cenocepacia serum we have established a protocol to increase bacterial internalisation within THP-1 macrophages enabling ~10-fold improvement in bacterial uptake using low multiplicities of infection. By improving bacterial uptake this dramatically improves the observable coverage of the B. cenocepacia proteome as well as the detection of changes within the host proteomes by minimising proteome dilution effects driven by high levels of uninfected host cells. By coupling opsonization with centrifugation-based isolation of intracellular B. cenocepacia we find comparable proteome coverage can be achieve to in vitro culture B. cenocepacia allowing the assessment of nearly 50% of the B. cenocepacia proteome during infection. Comparing early (3hr) and late (24hr) infections reveals distinct bacterial proteome profiles highlighting the importance of micronutrient acquisition during early infection.

Conclusion. Proteomics profiling of THP-1 macrophages infected using opsonization improves the identification of proteome changes during B. cenocepacia infections. By improving infection models of B. cenocepacia this work provides an new platform to understand the host-pathogen dynamics of B. cenocepacia within host cell models.

abs #049

Association of novel potential IgG3 allotype with malaria in children from Sepik region of Papua New Guinea

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Background. Malaria is a global health burden causing death and severe illness in children under five years of age. Recent work has established the importance of malaria-specific IgG3 in malaria immunity. Antibody allotypes due to single nucleotide polymorphisms (SNPs) of IgG3-Fc regions can modulate IgG3 Fc-mediated functions. A novel IgG3 potential allotype, G3m29, was recently reported in pregnant women from Sepik, Papua New Guinea, and has been shown to have enhanced affinity to FcγRIIa. We hypothesized that the presence of G3m29 provides protection from malaria in children.

Methods/Results. We used Sanger sequencing to study the sequences of the CH2 and CH3 domains of IgG3, in a cohort of children in the Sepik aged 1 to 3 years (N=203) whose number of malaria episodes were recorded over 18 months. Associations between exposure and outcomes were examined using linear regression. We identified SNPs and compared sequences to the references in the immunogenetics (IMGT) database. 78% of the cohort were either heterozygous (n=82, 40%) or homozygous (n=77, 38%) for the potential allotype G3m29. We also found a decrease in the total number of Plasmodium infections in children with potential G3m29 allotype compared to non-G3m29 allotype carriers (β= -1.736, 95% CI [-3.39, -0.079], p <0.05). This effect was most pronounced for P. vivax asymptomatic infections (β=-1.06, 95% CI [-2.01, -0.12], p <0.05). G3m29 carriers (N=167) had significantly lower levels of total IgG to Plasmodium vivax vaccine candidate proteins as compared to non-G3m29 carriers.

Conclusion. In conclusion, the potential G3m29 appears to confer protection against P. vivax infections.

abs #051

Association of Plasmodium falciparum specific afucosylated IgG with immune protective function activation

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Background. Malaria is a life-threatening disease that causes over 600,000 deaths annually, with the most severe form caused by Plasmodium falciparum. In terms of naturally acquired immunity, Immunoglobulin G (IgG) antibodies serve a critical role in activating immune-protective function via the Fc gamma receptors (FcγR). Recent focus on IgG fucosylation highlighted the heighted binding affinity of afucosylated IgG to FcγRIIIa compared to fucosylated IgG, due to the absence of fucose on the highly conserved N-linked glycan located in the Fc domain of IgG, resulting in enhanced antibody dependent cellular cytotoxicity (ADCC).

Methods/Results. In this study, we utilized the Fucose-sensitive Enzyme-linked immunosorbent assay (ELISA) for Antigen-Specific IgG (FEASI), an immunoassay that is capable of quantifying Fc fucosylation of antigen-specific IgG antibodies. FEASI consists of two ELISA assays; the first is to measure the levels of antigen-specific IgG independent to fucosylation using total IgG or N162A mutant FcγRIIIa receptor, while the second gives FcγRIIIa specific binding readouts which is highly sensitive to IgG fucosylation. The output of both ELISAs is converted into a ratio that represents levels of fucosylation in a given sample.

Here we examined the plasma from N=167 P. falciparum infected pregnant women of varying gravidities using FEASI. Our results showed varying levels of IgG fucosylation within the cohort and further experiments that explore the association between the levels of afucosylated IgG with neutrophil phagocytosis and NK cell activation are planned.

Conclusion. These results have important implications in the understanding of naturally acquired protection to malaria in pregnant women.

abs #059

Investigating immunity to non-pregnancy specific Plasmodium falciparum antigens and protection against placental malaria

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Background. Placental malaria, which is caused by Plasmodium falciparum results in adverse perinatal outcomes and is characterised by sequestration of infected erythrocytes (IEs) which bind to CSA. Antibody response to P. falciparum antigens have been reported to reduce parasitemia in the placenta and possibly contribute to protection. We sought to investigate if non-pregnancy specific antibodies contributed to protection against placental malaria.

Methods/Results. This study used plasma from pregnant Malawian women with parasitemia at antenatal booking, who either had placental infection (n=75) or had no placental infection (n=88) at delivery. Antibody levels of total IgG, IgG1-4, IgA1, IgA2, IgM and antibody engaging FcγRIIA, FcγRIIIA, FcγRIIB, FcγRIIIB and C1q to thirteen P. falciparum recombinant antigens which comprised eight merozoites (PfRh5, PfRh2a1, EBA175, MSP1-p19, MSP2, MSP3, MSP9, AMA1), antigens active in the schizont stage (PfSEA1-1A, PfGARP), vaccine candidate (PfCSP) and pregnancy specific antigens (VAR2CSA, DBL1-ID2a) were quantified by the Luminex multiplex assay. Levels of antibody features were compared using Welch's t test and a volcano plot was generated to visualize the distribution of antibody features between the two groups. In univariate analyses, 27 antibody features were more abundant in women with placental malaria with nineteen being antibody features to merozoites. Among five antibody features that were higher in pregnant women with no placental malaria were IgA1 antibodies to MSP2, MSP9, PfRh2a1, PfRh5 and complement binding antibodies to PfRh5.

Conclusion. Antibodies to merozoite antigens are primarily markers of exposure to malaria. Further analysis will include machine learning techniques to identify the antibody features that contribute to protection.

abs #090

Boosting TRM cell formation in the liver to combat disease

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Background. Memory T cells can be broadly classified into circulating (TCIRC) and tissue-resident memory T (TRM) cells. While TCIRC cells traffic through the blood, TRM cells reside within barrier sites, allowing them to mediate rapid protection against microbial insults and secondary exposures. Extensive heterogeneity exists across TRM cells depending on their tissue of residence. Development of CD103+ TRM cells in epithelial sites such as the skin and gut requires the coordination of the transcription factor Runx3 and the cytokine, TGFb. Conversely, TGFb-signalling is detrimental for TRM cell generation in the liver, yet it is unclear whether Runx3 is required for liver TRM cell development. We were therefore interested in understanding the role of Runx3 in liver TRM cell formation.

Methods/Results. T cells were transduced with retrovirus to ectopically express Runx3 and adoptively transferred into mice. We found that while high Runx3 expression increased TRM cell development in the skin, Runx3 overexpression reduced liver TRM cell development. Moreover, Runx3 diminished expression of the liver homing marker, LFA-1. Interestingly, enforced expression of Runx3 permitted TGFb sensitivity and generated epithelial-like CD103+ TRM cells.

Conclusion. While Runx3 is pivotal for epithelial TRM cell formation, high levels of Runx3 appears to be detrimental for TRM cell development in the liver. Instead, high Runx3 expression generated a subpopulation of epithelial-like CD103+ TRM cells in the liver. Since there are a subset of liver TRM cells in humans that express CD103, understanding the mechanisms as to how these cells are formed is critical to combat liver-specific diseases such as hepatitis.

abs #099

Understanding the expression and signalling properties of NKp44-isoforms in Natural Killer cell anti-tumour functions

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Background. Natural killer (NK) cells can restrict tumour growth by secreting proinflammatory cytokines and direct lysis of malignant cells. To achieve replicative immortality tumour cells often upregulate production of growth factors, including platelet-derived growth factor-D (PDGF-D). Intriguingly, PDGF-D binding to the NK cell receptor NKp44 triggers NK cell secretion of pro-inflammatory cytokines, but whether this extends to cytotoxicity is unknown. Moreover, how the three NKp44-isoforms regulate NK cell activity is controversial. NKp44 isoform-1 (NKp44-1) encodes a cytoplasmic tyrosine-based sequence that predicts inhibitory signalling properties. Absent NKp44 isoform-specific antibodies it has been difficult to answer questions of the signalling mechanisms and relative expression of these proteins. Here, we characterise and employ novel NKp44-isoform antibodies to decipher the functional differences of NKp44-isoforms in NK cell anti-tumour immunity.

Methods/Results. We adapted a flow cytometry-based cytotoxicity protocol to investigate the effect of PDGF-D on NK cell cytotoxicity. We report that NK cells kill tumour cell more efficiently in the presence of PDGF-D. Next, we generated GFP reporter cells genetically engineered to express each NKp44-isoform. We show that all NKp44-isoforms activate green fluorescent protein reporter cells upon binding to PDGF-D. Indeed, lower surface expression of NKp44-1 suggests that the tyrosine-based cytoplasmic motif may mediate receptor endocytosis. Furthermore, using these reporter cells we validated the specificity of novel anti-NKp44 antibodies and show that these proteins are expressed in NK cells.

Conclusion. This work provides novel descriptions of the expression and signalling pathways of NKp44-isoforms and evidence that PDGF-D can trigger NK cell cytotoxicity of tumour cells through NKp44.

abs #108

Methylseleninic acid induces HIV viral reactivation from latently infected cells in vitro and ex vivo

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Background. HIV continues to be a pathogen of global importance, with 40 million people currently living with the virus. Preventing cure is the reservoir of latently infected cells which persist despite antiretroviral therapy (ART). The 'shock and kill' approach to HIV cure relies on reversing latency and inducing death of infected cells. Here we investigate the organic selenium compound Methylseleninic acid (MSA) as latency reversal agent (LRA), alone and in cotreatment with previously characterised LRAs.

Methods/Results. In latently cell lines containing an integrated HIV provirus with a green fluorescent protein (GFP) reporter (J-LAT10.6), MSA induced a 23.12-fold increase in GFP expression; In synergy studies with other LRAs, MSA synergised (Bliss Independence, BI) with the bromodomain inhibitor, JQ1 (BI = 0.09, p=0.0418) and SMAC mimetic, AZD5582 (BI = 0.5, p=0.0109) in viral reactivation. In CD4 T-cells isolated from people living with HIV (PLHIV), MSA activated viral transcription (4.4-9-fold increase over untreated in unspliced HIV RNA); induced changes in cell activation markers (6.8-52.5 fold-increase in CD69 expression and 4.21-39.6 fold-decrease in CD38 expression); and significantly upregulated pro-apoptotic gene expression (SMAC, BIM, BAK1) and downregulated anti-apoptotic gene expression (XIAP, BCL2) measured via bulk RNAseq.

Conclusion. MSA induced HIV reactivation in latently infected cell lines, and in CD4+ T cells from PLHIV in the absence of sustained T-cell activation. Interestingly, MSA treatment also led to a pro-apoptotic phenotype suggesting it may induce cell death in HIV-infected cells alongside its latency reversal function. MSA demonstrates promise as a dual 'shock and kill' agent.

abs #111

The expression of BCL-2 family members in conventional and unconventional T Lymphocyte subsets

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Background. Apoptosis, a type of programmed cell death used by multicellular organisms, is vital for human development and immune system function. Two distinct pathways, extrinsic and intrinsic, cause apoptosis. DNA damage, hypoxia, and metabolic imbalance activate the intrinsic pathway. The primary pathway regulator is the BCL-2 protein family. Some proteins in this family promote apoptosis, whereas others hinder it, and a cell's fate is influenced by the ratio of these pro- and anti-apoptotic BCL-2 family members. Since T cell death mechanisms are likely to vary by subpopulation, we examined BCL-2 family member expression in various subsets of T cells from mice.

Methods/Results. Spleens and lungs were harvested from mice and were analysed to identify T cell subsets, and BCL-2, BCL-XL, and BIM expression. The expression of BCL-2 family members was found to vary among T cell subsets. While central memory CD8+ T cells in the spleen exhibit higher expression BCL-2 than other cell subsets, natural killer T (NKT) cells exhibit higher BCL-XL expression, and mucosal-associated invariant T (MAIT) cells demonstrate higher BIM expression. In the lung, BCL-2, BCL-XL, and BIM are expressed at higher levels by central memory CD8+ T cells, effector memory CD8+ T cells, and MAIT cells, respectively.

Conclusion. Expression of BCL-2 family members varies between T cell subsets and remains largely uncharacterised in unconventional T cells. As unconventional T cells are a potential target for cancer immunotherapy and vaccines against pathogens, understanding the BCL-2 family's expression patterns may provide insights into the design and development of more effective vaccines and immunotherapies.

abs #119

Partners in crime - investigation of influenza-group A streptococcus coinfection

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Background. The upper respiratory tract harbors a diverse community of microbes including the bacterium group A streptococcus (GAS) and influenza virus. Emerging epidemiological evidence suggests an association between influenza coinfection and severe cases of GAS disease. However, little is known about influenza-GAS interactions.

Methods/Results. In this study, we examined influenza-GAS coinfection by establishing an air-liquid interface model using primary human nasal epithelial cells. When fully differentiated, the air-liquid interface model includes mucous-producing and ciliated cells, closely resembling the respiratory epithelium in vivo. Using this model, we determined the effect of influenza A virus (subtype H3N2) on GAS and the coinfected respiratory epithelium. Prior influenza infection did not affect bacterial load immediately after bacterial inoculation but increased the density of several GAS strains by approximately 100-fold at 24 hours post-bacterial infection ($n=20$, $p<0.0001$). Coinfection also led to a higher proportion of intracellular bacteria ($n=7$, $p=0.03$), measured by antibiotic protection assay. Additionally, influenza-GAS coinfection reduced transepithelial electrical resistance ($n=4$) and cilia beating frequency ($n=4$-5) compared with GAS ($p=0.004$ and 0.1, respectively) or influenza ($p=0.003$ and 0.02, respectively) mono-infections.

Conclusion. This study presents novel experimental data demonstrating that influenza coinfection promotes GAS replication in the upper respiratory tract but not initial bacterial adhesion to nasal epithelial cells. These results suggest that influenza virus may have a role in facilitating GAS dissemination within the host. Future studies will examine the effect of influenza on the bacteria and host to understand the biological processes involved.

abs #126

Cytoplasmic material transfer from conventional dendritic cells to marginal zone B cells during trogocytosis

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Background. Trogocytosis is a cellular process in which one cell extracts membrane fragments and membrane-embedded molecules from another cell, with the possibility of incorporating these fragments to its own membrane. Notably, trogocytosis has been observed between marginal zone B cells (MZBC) and conventional dendritic cells type 1 (cDC1) when the complement component C3 binds to the carbohydrate moiety of peptide-MHC II (pMHC II) on the cDC1 surface. Upon binding, MZBC recognise the cleaved form, C3dg, through their CR2 receptor, subsequently initiating the transfer of membrane to MZBC. Previous reports have indicated that MZBC acquire pMHC II and surface molecules such as XCR1 or CD8 through trogocytosis. In this study, our objective was to explore whether cytoplasmic contents were also transferred.

Methods/Results. To investigate this matter, splenic dendritic cells were isolated from mice wherein the Venus fluorescent protein was encoded in the XCR1 locus, making cDC1 cells Venus-fluorescent. The cytoplasmic localisation of Venus was confirmed by confocal laser scanning microscopy. B cells were purified from mice that lacked Venus expression. Subsequently, these cells were co-cultured for 2 hours, followed by flow cytometry analysis. The results revealed that a considerable fraction of MZBC displayed detectable levels of the fluorescent protein.

Conclusion. This study uncovers a novel aspect of trogocytosis between cDC1 and MZBC, suggesting that cytoplasmic contents are transferred during trogocytosis as well as membrane and membrane-anchored molecules. Further research into the underlying mechanisms governing this transfer may shed light on the functional implications of trogocytosis between cDC1 and MZBC.

abs #127

Glycoproteomic analysis of Acinetobacter baumannii reveals PglLAB to be a serine specific oligosaccharyltransferase

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Background. Protein glycosylation is a ubiquitous process observed across all domains of life. Recently glycoproteomics tools have seen dramatic improvements in performance yet many forms of protein glycosylation remain refractory to analysis such as bacterial glycosylation. Once thought absent in bacterial systems protein glycosylation is now known to be widespread yet utilises diverse carbohydrates not synthesized within eukaryotic systems. These differences in the glycan compositions have the potential to impact all steps within glycoproteomics workflows including digestion, enrichment, and analysis requiring optimisation to ensure optimal glycoproteomic performance. The nosocomial pathogen Acinetobacter baumannii has been previously shown to possess a general O linked glycosylation system essential for biofilm formation and virulence yet the substrates and function of this system are poorly understood.

Methods/Results. To expand our knowledge of the substrates of A. baumannii glycosylation we assessed the performance of glycoproteomics approaches for the characterisation of three A. baumannii strains possessing diverse glycan structures ATCC19606, BAL062, and D1279779. We demonstrate the value of incorporating glycan-specific diagnostic ions to enhance data collection of specific glycoforms leading to improve glycan localization. Utilising these tailored data collection methods coupled with different glycopeptide enrichment approaches and the use of multiple proteases we expand the coverage of the A. baumannii glycoproteome revealing 31 glycoproteins and mapping glycosylation to 47 serine residues including 36 novel glycosylation sites with the specificity of A. baumannii PglL for serine residues confirmed through molecular approaches.

Conclusion. Combined this research significantly expands the current knowledge and site specificity of the A. baumannii glycoproteome.

abs #130

Mechanisms underlying perturbed NK cell activation during pregnancy

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Background. Respiratory infections such as those caused by SARS-CoV-2 and influenza viruses are of major concern for human health. While most otherwise-healthy individuals do not succumb to infection, there are groups at increased risk of disease severity, including pregnant women. The mechanisms for severe respiratory infection during pregnancy are currently not well understood. To address this, our recent work (Habel et al. 2023, JCI Insight) comprehensively analysed cellular activation, SARS-CoV-2-specific antibodies, and inflammation in pregnant and non-pregnant women with COVID-19. Notably, we identified perturbed NK cell activation dynamics during pregnancy, but it remains unknown whether this is a correlate of protection or disease severity.

Methods/Results. To identify the mechanisms driving increased NK cell activation during pregnancy, >350 proteins were screened on NK cells from pregnant and non-pregnant women using a flow cytometry approach. Differentially expressed proteins in NK cells during pregnancy related to cell activation, adhesion, and lipid metabolism. To determine whether these differences were also reflected at the transcriptional level, single-cell RNA sequencing was performed. Gene ontology analysis of 361 differentially expressed genes in NK cells from pregnant and non-pregnant women revealed an upregulation of genes involved in cell activation during pregnancy.

Conclusion. Having identified pathways contributing to perturbed NK cell activation during pregnancy, future functional assays will demonstrate their involvement in anti-viral immunity. Overall, our study finds that pregnancy impacts NK cell immunity and provides insight on potential targets to prevent severe respiratory infections during pregnancy.

abs #134

Redefining iNKT cells thymic development pathway in human

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Background. iNKT cells, a key subset of unconventional T cells, play an important role in the immune system. Understanding how these cells develop is crucial to harnessing them for therapeutic purposes. While many studies have examined iNKT cells from mice, relatively few have examined iNKT cells from the human thymus. The purpose of this study was to identify the thymic development pathway of iNKT cells in humans.

Methods/Results. The expression pattern of PLZF was chosen as a guide because PLZF plays a key role in mouse iNKT cell development. In addition to staining human thymus samples for PLZF, we also stained them for markers of T cell maturation and function. This pathway was then validated using a functional cytokine assay. As we expect the most mature cells in the blood, we checked the development pathway in donor-matched blood as well. We have identified a four-stage pathway of development for iNKT cells in the human thymus. stage 1 cells were low for PLZF expression, stage 2,3 iNKT cells were medium, and stage 4 were the highest. Also upon stimulation, iNKT cells started to produce TNFa from stage 2, IL-2 from stage 3, and IFg from stage 4. Blood samples only contained stage 3 and 4 cells.

Conclusion. These data reveal distinct precursors of iNKT cells expressing PLZF and other maturation markers within thymic development. These findings may lead to new opportunities to manipulate iNKT cells to treat human diseases, including cancer, inflammation, and infection.

abs #138

Role of IgG antibodies in protection from placental malaria birth outcomes

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Background. Placental malaria (PM) is a public health issue linked to poor pregnancy outcomes. Antibodies against VAR2CSA, a variant surface protein found on infected erythrocytes, protect against Plasmodium falciparum infections in pregnant women. This study aims to associate VAR2CSA antibody levels with poor pregnancy outcomes: Low Birthweight (LBW), preterm delivery, Small for Gestational Age (SGA) and maternal anaemia.

Methods/Results. Pregnant Malawian women (n=466) infected with malaria were recruited at 16-28 gestation weeks. Total IgG levels to recombinant VAR2CSA (DBL1X-ID2a) domains were measured at enrolment and delivery via indirect ELISA. Results show significantly higher IgG levels in multigravid women (3.50 ± 0.10 AU) than primigravid women (3.38 ±0.10 AU). High antibody levels were observed at enrolment (3.45 ±1.09 AU) compared to delivery (2.89 ± 0.97 AU). However, IgG antibody levels measured at enrolment were not associated with reduced LBW (aOR=1.16, 95%CI 0.81-1.67, p= 0.4), preterm delivery (aOR=1.24, 95%CI 0.81-1.88, p=0.32), SGA (aOR=0.96, 95%CI 0.74-1.25, p=0.75) and maternal anaemia (aOR=1.08, 95%CI 0.84-1.40, p=0.55). This shows no significant associations between the antibody levels at enrolment with protection from poor pregnancy outcomes.

Conclusion. The antibody responses to VAR2CSA are likely markers of PM rather than protection from infection.

abs #139

Exploring the role of type I interferon in β-glucan-mediated reprogramming of haematopoietic stem cells

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Background. Immune memory is a defining feature of the adaptive immune system, but activation of innate immune cells can also result in a heightened response to re-challenge. This innate immune memory-like adaptation has been termed “trained immunity”. β-glucan (a fungal cell wall component) is one of the best-studied triggers of trained immunity and was recently found to act on the haematopoietic stem cells (HSCs) in the bone marrow (BM) to promote the generation of myeloid cells with heightened “pro-inflammatory” potential. However, the underlying mechanism leading to these β-glucan-mediated haematopoietic changes is incompletely understood.

Methods/Results. We used mouse models of β-glucan-induced trained immunity to demonstrate that type I interferon (IFN) is responsible for the β-glucan-mediated reprogramming of BM HSCs and the expansion of progenitor populations. Following β-glucan treatment, IFNα levels were elevated in the BM and increased in BM plasmacytoid dendritic cells, type I IFN receptor (IFNAR1) levels were enhanced on HSCs, and the frequencies of multipotent progenitor (MPP) subsets and granulocyte-macrophage progenitors (GMPs) were expanded. Blockade of endogenous type I IFN signalling with a neutralising antibody against IFNAR1 suppressed the β-glucan-driven increases in mitochondrial activity and cell cycle progression in HSCs while preventing the expansion of MPPs. Only the myeloid-biased MPP3 subset showed a sustained expansion 7 days after the initial β-glucan administration, which was lost upon co-delivery of anti-IFNAR1 neutralising antibodies.

Conclusion. We find that IFNα signalling plays a key role in the β-glucan-mediated reprogramming of HSCs and the expansion of myeloid-biased progenitor MPP3s, which are integral components of β-glucan-mediated trained immunity.

abs #153

Development and characterisation of a SARS-CoV-2 RNA vaccine expressing three linked-RBD domains

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Background. A novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for the ongoing COVID-19 pandemic. While the leading vaccines such as BNT162b2 and mRNA-1273 make use of mRNA-based technology, the unmodified self-amplifying mRNA (SAmRNA) vaccine platform uses low doses and has self-replicative properties conferred by the alphavirus replicase genes. The aim of our study is to investigate COVID-19 RNA vaccines expressing three different linked-receptor binding domains (RBDs) using either a typical modified mRNA or an alphavirus SAmRNA.

Methods/Results. The 3RBD vaccine antigen we designed tethered together the RBDs of Beta, Delta and BA.1 variants with a short flexible linker, incorporating a C-terminal transmembrane domain. The plasmid template was optimized for cap, codons, poly(A) and pseudouridine for maximum mRNA expression. We compared the same antigen expressed from native structured RNA in a Venezuelan equine encephalitis virus-derived (VEEV) SAmRNA expression vector.
The 3RBD vaccine antigen was robustly expressed in vitro from either a typical mRNA or VEEV SAmRNA. Successful binding of 3RBD to different classes of human monoclonal antibodies and human ACE2 receptor was observed via flow cytometry. Encapsulated RNA lipid nanoparticles versions were made to optimal size, polydispersity and encapsulation efficiency.

Conclusion. We were successful in producing two alternate versions of a booster RNA COVID vaccine and have validated the antigenicity of our novel polyvalent 3RBD antigen. Our research shows promising results for the development of a high neutralisation breadth yet RBD focused SARS-CoV-2 vaccine antigen to minimise effects of imprinting that is proceeding to further investigation for protective efficacy in vivo.

abs #155

Unveiling the interplay of Coxiella burnetii Dot/Icm effectors: insights into host immune subversion

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Background. Coxiella burnetii, the causative agent of human Q fever, is a unique bacterial pathogen that obligatorily replicates within a lysosome-derived intracellular space. Central to the establishment of this niche and ensuing pathogenesis is the Dot/Icm type IV secretion system encoded by the pathogen. This system translocates approximately 150 distinct effector proteins directly into the host cytosol, with several playing indispensable roles in bacterial virulence by subverting various host cellular processes to enable C. burnetii intracellular proliferation. However, the functional roles of most of these effectors remain undefined.

Methods/Results. We harnessed budding yeast as a practical heterologous system to query the functions of C. burnetii effectors within a eukaryotic cellular context. Analysing the impact of each effector ectopically expressed in Saccharomyces cerevisiae marked 11 as ‘toxic’ due to their suppression of yeast growth, including a nuclear-targeting effector termed NceA (nuclear Coxiella effector protein A). A yeast toxicity suppressor screen identified nuclear activities, including regulation of gene transcription, chromatin remodelling, and ribosome biogenesis, as subverted by NceA. A further system-wide screen using a modified Synthetic Genetic Array approach discovered two other effectors capable of alleviating NceA-induced yeast toxicity, indicating them as potential regulators of NceA host cellular functions (RonA and RonB) during infection. Further validation in tissue culture infection models demonstrated an antagonistic functional interplay between NceA and RonA, influencing the activation of NF-κB in response to C. burnetii infection.

Conclusion. This study advances our comprehension of C. burnetii effectors and their intricate coordination in undermining host immune signalling to ensure successful infection.

abs #166

Identifying new roles for E3 ligases in regulating dendritic cell function.

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Background. Ubiquitination is a post-translational modification that regulates protein degradation and activity. E3 ubiquitin ligases are key enzymes that catalyse the addition of ubiquitin to target proteins. Dendritic cells are important immune cells that function as antigen-presenting cells, linking the innate and adaptive arms of the immune system. This project aimed to identify new E3 ubiquitin ligases and associated scaffold proteins that may play a role in regulating dendritic cell function.

Methods/Results. An arrayed CRISPR/Cas9 knockout screen covering all known and putative E3 ligases and scaffold proteins was performed in a Cas9+ DC1940 dendritic cell line. Following transduction of the DC1940 cells with lentivirus encoding a single guide RNA, cells were cultured for six days before being split into resting dendritic cells or stimulated with TLR9 ligand CpG and interferon γ. The effect of gene knockout on dendritic cell function was assessed by measuring the expression of key functional surface markers by flow cytometry. The screen identified five potential regulators of dendritic cell function in the resting state: Ariadne RBR E3 ubiquitin protein ligase 1 (Arih1), scaffold protein cullin3 (Cul3), zinc finger and BTB domain-containing protein 14 (Zbtb14), ring finger protein 31 (Rnf31), and RAB guanine nucleotide exchange factor 1 (Rabgef1). Further characterisation revealed Arih1 and Cul3 as regulators of MHCI, CD80, CD86, Flt3, Clec9a and DEC205 expression, and Zbtb14 as a regulator of Flt3 surface expression.

Conclusion. Arih1, Cul3, Rabgef1, Rnf31 and Zbtb14 were identified as potential regulators of dendritic cell function.

abs #169

Understanding neuropathogenesis in cases of viral respiratory infections

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Background. Neurological symptoms have been observed in cases of respiratory viral infections. This study examines the cellular and viral mechanisms which drive neuropathogenicity in respiratory viral infections. It is hypothesized that virus-specific activation of NOD-like receptor protein 3 (NLRP3) inflammasome determines the neurological outcome of respiratory viral infections. In vitro and in vivo studies were performed to elucidate the neurological consequences of infection with high and low-virulence influenza A virus strains.

Methods/Results. Peripheral macrophages and neural cells (including microglia, neurons, and astrocytes) were exposed to nigericin, an activator of the NLRP-3 inflammasome and peptides derived from high-virulence and low-virulence influenza A virus (IAV) strains to determine NLRP3 inflammasome activation. Macrophages produced high levels of IL-1b in response to treatment with nigericin and peptides derived from high virulence IAV strain.
Wild-type C57/BL6 and NLRP3-/- mice were infected with high virulence IAV. Viral titre indicated that both groups were experiencing productive infection however weight change indicated that NLRP3-/- mice experienced the more severe disease. Brains were collected to investigate neuroinvasion and neuropathogenesis in these models of mild and severe IAV infection.

Conclusion. This study has established that peptides derived from highly virulent strains of IAV are capable of activating the NLRP3 inflammasome. Mice lacking the NLRP3 inflammasome experience more severe disease and this will be investigated in the context of neurological disease.

Mental Health and Psychosocial Research

abs #011

Young men, suicide and relationship breakdown: A systematic review and meta-analysis

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Background. Intimate relationship breakdown represents both a predisposing and precipitating risk factor for suicidal thoughts, behaviours, and death in men. Yet while relationship breakdown is a common experience, we know little about the factors explaining which men are most vulnerable to suicidality following relationship breakdown. This systematic review and meta-analysis therefore aimed to synthesise the available evidence for diversity in pathways to suicidality following relationship breakdown in men.

Methods/Results. Seven electronic databases were searched with terms related to men, relationship breakdown, and suicidality. Data were extracted from each article, forming a narrative synthesis of findings with accompanying random-effects meta-analysis of the pooled odds of suicidal thoughts, attempt and death following relationship breakdown in men.

A systematic search yielded 3,358 records, where 73 met inclusion criteria, and 30 had sufficient data for meta-analysis. Findings highlight the role of sociodemographic factors (e.g., age, education), characteristics of relationship breakdown (e.g., recency of breakdown; ex-partner-initiated separation) and psychosocial factors (e.g., loneliness, substance misuse, shame) in amplifying men's vulnerability to suicidality following relationship breakdown. Meta-analyses found elevated odds of suicidal ideation (OR=1.64 [1.27, 2.13]); attempt (OR=1.73 [1.05, 2.85]); and death (OR=2.69 [2.33, 3.11]) among divorced relative to married men. Separated men aged under 35 displayed eight times the risk of suicide than their married counterparts (OR=8.79 [4.32, 17.69]).

Conclusion. Findings highlight several viable intervention targets to prevent suicide among recently-separated men who experience markedly elevated suicide risk. There was nevertheless a lack of prospective data substantiating variability in pathways to, and psychosocial adjustment following, relationship breakdown in men.

abs #024

Cardiometabolic disease risk factors and cognitive function in bipolar disorder: Findings from the UK Biobank

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Background. Cardiometabolic disease risk factors are disproportionately prevalent in bipolar disorder (BD) and are associated with cognitive impairment. Despite this, the extent to which health risk behaviours compared to physiological risk factors, are more, less, or equally relevant, remains unknown. This study aimed to identify which cardiometabolic disease risk factors are the most important correlates of cognitive impairment in BD; and to determine whether the nature of the correlations vary between mid and later life.

Methods/Results. Data from the UK Biobank were available for 966 participants with BD. Individual cardiometabolic disease risk factors were regressed onto a score of global cognition within three risk factor domains (physiological: anthropometric and clinical, physiological: cardiometabolic disease risk biomarkers, and health risk behaviours). A final combined multivariable regression model for global cognition was then fitted, including only the predictor variables that were significantly associated with cognition in the previous models.

In the final combined model, decreased mentally-active and increased passive sedentary behaviour, high levels of physical activity, inadequate sleep duration, increased systolic and decreased diastolic blood pressure, and decreased handgrip strength were significantly associated with global cognitive impairment. Waist circumference and CRP were significantly associated with cognition in the domain specific models only.

Conclusion. These findings provide preliminary evidence suggesting that cardiometabolic disease health risk behaviours, as well as blood pressure and muscular strength, contribute to cognitive function in BD, whereas physiological cardiometabolic disease risk largely does not.

abs #030

Parent Emotion Socialization is Associated with Neural Correlates of Emotion Regulation in Early Adolescents

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Background. Early adolescence is a developmental period marked by significant biological and social-emotional changes, and is also a time of heightened vulnerability to emotion regulation difficulties. During this period, neural networks supporting emotion regulation undergo dynamic alterations, rendering early adolescents particularly sensitive to environmental influences. Parent emotion socialization behaviors play a critical role in shaping the healthy development of emotion regulation in young people; however, the impact of such behaviors on the neural correlates of emotion regulation in early adolescents is not well understood. In this study, we aimed to examine the association between parent emotion socialization and neural activity during emotion regulation tasks in early adolescents.

Methods/Results. Methods: Participants were 47 female adolescents aged between 10 to 12 years. Adolescents reported on their parents' emotion socialization behaviors and performed two fMRI tasks: an affect labeling task (implicit emotion regulation) and a cognitive reappraisal task (explicit emotion regulation). We performed both hypothesis-driven region of interest (prefrontal cortex [PFC], amygdala) analyses, in addition to exploratory whole-brain analyses, to investigate associations between supportive and unsupportive parent emotion socialization behaviors and adolescent brain function during emotion regulation.

Results: Supportive parent emotion socialization behaviors were associated with greater activation in the dorsomedial and ventromedial PFC (dmPFC, vmPFC) and dorsal anterior cingulate cortex (dACC) during implicit emotion regulation (affect label vs shape label). Unsupportive emotion socialization behaviors were associated with less activation in the dmPFC, vmPFC, and right hippocampus during implicit emotion regulation. Parent emotion socialization was not associated with neural activation during explicit emotion regulation (cognitive reappraisal vs passive viewing of negative pictures).

Conclusion. Findings from this study suggest that both higher levels of supportive emotion socialization (e.g., validating children's emotions) and lower levels of unsupportive emotion socialization (e.g., dismissing and punishing emotions) may influence emotion regulation related brain function in early adolescents. Associations with brain function during implicit but not explicit emotion regulation may indicate that neural infrastructure underlying explicit emotion regulation typically develops throughout adolescence. As such, early adolescents may vary in their ability to employ cognitive reappraisal, which may explain why we did not observe any significant association between parent emotion socialization and neural activity during explicit emotion regulation.

abs #033

Exploring emotional biases during inhibitory control performance: Insights from an emotional antisaccade task in patients with bipolar disorder

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Background. Bipolar disorder (BD) is a pervasive psychiatric condition associated with mood dysregulation and trait-like impairments in cognition, and particularly in inhibitory control. To investigate inhibitory control deficits in BD, we used eye-tracking to measure performance on antisaccade tasks with both emotional "hot" and non-emotional "cold" valenced stimuli. The task included two types of trials: step trials, without a gap prior to stimulus presentation, and gap trials, which included a 200ms gap.

Methods/Results. Results from 40 euthymic BD patients and 27 controls indicated no significant group differences for neutral, positive or negatively valenced stimuli. Overall, participants from both groups demonstrated worse performance on neutral stimuli compared to emotional stimuli. Participants from both diagnostic groups also showed faster responses and lower error rates on gap trials compared to step trials, indicating that they found it easier to disengage attention during gap trials.

Unexpectedly, during the more attentionally-demanding step trials the BD group exhibited faster responses on positively valenced stimuli compared to controls, but showed no difference in error rate.

Conclusion. This finding indicates that BD patients may find it easier than controls to attentionally disengage from positively valenced stimuli during more challenging conditions.

These findings may provide insight into the effect of emotional biases and attentional disengagement on antisaccade performance, and highlight the importance of examining both hot and cold components of inhibitory control in BD.

abs #042

Cognitive and psychological presentation prior to CAR-T therapy: a real-world approach.

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Background. Immune effector cell-associated neurotoxicity syndrome (ICANS) is a common consequence of chimeric antigen receptor T-cell (CAR-T) therapy, with a wide range of neurocognitive presentations. Patients are rarely examined prior to CAR-T, complicating the distinction between new symptoms and pre-existing dysfunction. The study aimed to characterise baseline cognitive and psychological status of haematology patients planned for CAR-T.

Methods/Results. Sixty patients underwent a specialist cognitive assessment prior to receiving CAR-T at Peter MacCallum Cancer Centre. Data were obtained from clinical examinations, psychometric measures, and a self-report questionnaire of psychopathology and subjective cognitive function. A subset of patients completed a screening measure of cognition to examine its utility in identifying impairment.
According to the clinician's impression or to a purely psychometric approach, 15-16 (25.0%-27.0%) patients presented with evidence of cognitive impairment, with six unique patterns of dysfunction. Of those patients who completed a self-report measure of psychopathology, nine (15.8%) were elevated on at least one domain. The differences in clinicodemographic characteristics between cognitively impaired and cognitively normal individuals were analysed using the Bayes factor (BF10). Cognitively impairment patients were more likely to have B-cell acute lymphoblastic leukaemia (BF10=9.30), be younger (BF10=7.76), have bone marrow involvement (BF10=5.18), report history of anxiety (BF10=4.85), or have evidence of psychopathology (BF10=31.30). Screening approaches were not useful in detecting impairment.

Conclusion. The study demonstrated a broad spectrum of cognitive dysfunction and psychopathology in haematology patients prior to CAR-T. Baseline specialist cognitive evaluation is important for detection and management of cognitive neurotoxicity symptoms that might arise after the infusion.

abs #048

Characterising intra-individual cognitive fluctuations in bipolar disorder; brain, behaviour and beyond

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Background. Cognitive impairment is a core symptom of bipolar disorder (BD), but its behavioural and biological basis is unclear. A relevant factor in neurological conditions featuring similar cognitive symptomatology to BD is intra-individual variability (IIV). IIV describes the within-person fluctuation in performance over the course of a cognitive task, and it has not yet been researched in BD. This study sought to characterise IIV in BD; exploring if its increased in patients, investigate if it's related to cognitive impairment, and probe its associated neurobiology.

Methods/Results. Methods: Two hundred and seventeen adults (n=100 BD; n=117 healthy controls) participated, with IIV operationalised as each individual's standard deviation in reaction time on a repeated-measures attentional task. Cognitive tests of processing speed, memory, and executive function were also administered. A subsample of 55 BD participants underwent diffusion tensor neuroimaging to investigate white matter microstructure. Data were analysed using ANOVA and Pearson's correlation.

Results: Despite being matched on age and premorbid intelligence, BD participants had significantly more IIV than controls. IIV was significantly associated with cognition in BD alone, with greater IIV conferring poorer performance across all tasks. IIV also correlated with microstructural measures of fractional anisotropy and radial diffusivity.

Conclusion. BD participants were more cognitively inconsistent, with these fluctuations negatively associating with not only their overall functioning but also measures of reduced microstructural integrity and increased demyelination. Taken together, these findings suggest that a lack of integration in the brain structural network could facilitate inconsistencies in BD functioning. Future work disentangling causative patterns amongst these variables is now essential.

abs #057

The effects of social isolation stress on compulsive- and anxiety-like behaviour in a mouse model of relevance to obsessive-compulsive disorder

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Background. Obsessive-compulsive disorder (OCD) is a prevalent and debilitating mental illness that is frequently refractory to the best available evidence-based therapies. Despite extensive preclinical and clinical studies, our understanding of the genetics of this disorder remains incomplete. Furthermore, only limited research has focused on gene-environment interaction effects in the context of OCD and related disorders, despite the known role of stress as both an antecedent and maintaining factor in obsessive-compulsive symptomology.

Methods/Results. Wild-type (WT) and SAPAP3 knockout (KO) mice (a genetic model of relevance to OCD) were kept in either standard-housing (SH) conditions or socially isolated (SI) from five weeks of age. We assessed compulsive- and anxiety-like behaviour at the commencement of SI, and after both 3 and 6 weeks of SI. We found that chronic SI during adolescence increased grooming bouts in WT and KO mice, and had a sex-specific effect to increase time spent grooming only in male KO animals. We also found that acute SI caused a sex-specific increase in anxiety-like behaviour in male KO mice.

Conclusion. Congruent with our hypotheses, we found that SI stress increased compulsive- and anxiety-like behaviour in male SAPAP3 KO mice. Our data is the first to show the role of sex-specific gene-environment interactions in a mouse model of relevance to OCD, and we speculate that these effects may be due to sexually dimorphic pathogenic mechanisms that are sensitive to environmental influence. Importantly, our findings also suggest that more attention should be paid to sex differences in preclinical studies of OCD.

abs #060

The guru in your pocket: Findings from a cross-sectional study on characteristics of aspiring, engaged and disengaged meditation app users

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Background. Meditation apps are an extremely accessible form of meditation resource, and are downloaded by millions of people annually. However, these apps see large, early drop-offs in engagement of new users, sometimes exceeding 95%. Limited research has focused on factors that drive continued engagement and factors that may contribute to discontinuation of use. In studies that do report on reasons for drop out, findings rely on a single item. The current study will investigate what factors are associated with engaged current or retrospective app use. This cross-sectional study will survey individuals who have previously meditated or intend to start meditating using an app. The survey will gather information regarding the association between engagement and factors including demographics, app features, user characteristics and motivations to practice. The study will identify key predictors and sample numbers for a subsequent longitudinal study on sustained meditation app engagement in novice meditators.

Methods/Results. Cross-sectional survey is being conducted, associations will be identified via regression.

Conclusion. TBD - survey is currently being conducted

abs #068

Adverse childhood experiences and gender diversity: A comparison of cis- and transgender adolescents in a community sample.

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Background. Increases in the number of transgender youth presenting to gender services globally has led to interest in understanding factors related to the development of gender diversity. Some have claimed that transgender young people are exposed to higher levels of adverse childhood experiences (ACEs) and proposed that ACEs may play a role in identifying as transgender, but substantial methodological flaws in the underlying research cast doubt on such claims. The aim of our study was to use a population representative sample to examine the relationship between ACEs and gender diversity.

Methods/Results. We used the Adolescent Brain Cognitive Development study, a population representative study of US adolescents to compare the lifetime occurrence of ACEs in cisgender (n=5945) and transgender (n=85) adolescents aged 11-14. We calculated odds ratios (OR) for identifying as transgender based on exposure to nine common ACEs, including physical and sexual abuse, emotional and physical neglect, divorce/separation, and household violence, alcohol abuse, mental illness and involvement in the criminal justice system. We identified varying levels of ACEs among cis and trans adolescents. Across the nine ACEs assessed, we found insufficient evidence of a difference in the likelihood of identifying as transgender on the basis of ACEs exposure with the exception of having a parent with alcohol-related problems where a minor increase in odds was noted (OR 1.12; 95% CI 1.12-1.12)

Conclusion. Our study provides minimal evidence for the theory that exposure to ACEs during childhood drives the development of gender diversity during adolescence, but further longitudinal research is needed.

abs #072

Subcortical networks underlying the processing of negative beliefs related to the self, food, and body image

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Background. Repetitive negative thinking surrounding food, body image and the self often precipitates disordered eating behaviours. As such, psychotherapy for eating disorders frequently involves restructuring these maladaptive cognitions. Despite its clinical relevance, the neurobiological substrates underpinning cognitive restructuring remain unclear.

Methods/Results. Combining ultra-high field 7-Tesla magnetic resonance imaging and a novel cognitive restructuring paradigm, we examined changes in neural activation during the repeating or challenging of negative self- and eating-related beliefs in 48 healthy adults.

Challenging negative beliefs relative to repeating negative statements elicited increased activation in frontostriatal cognitive control regions, including the pre-supplementary motor area and the caudate. Whereas repeating negative beliefs was associated with heightened activity in default mode network regions linked to self-related processes. Interestingly, we identified that habenula activity – small midbrain nuclei implicated in negative event processing and the regulation of food intake – was modulated by cognitive restructuring and repetitive negative thinking.

Conclusion. This study identified key cortical and subcortical regions involved in the processing of negative beliefs. Notably, we found the first evidence to demonstrate habenular involvement in processing negative cognitions, laying the foundation for future work to examine potential habenula dysfunction in people with disordered eating.

abs #078

Investigation of the association between estradiol levels and brain structure and function in early adolescent females.

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Background. Sex difference in internalising disorder rates may be related to pubertal factors, such as the influence of pubertal hormones on brain development. There is a need to investigate the associations between female sex hormone (e.g., oestradiol) levels and both brain structure and function in adolescents, particularly during early adolescence when hormone levels begin to surge.

Methods/Results. Using Adolescent Brain Cognitive Development (ABCD) imaging and hormone data (N = 2204 females, aged 9-12 years), we used elastic-net regression to investigate associations between brain features (gray matter volumes, cortical thickness, surface area, sulcal depth, and white matter microstructure, resting-state connectivity, emotional n-back task-related function) and oestradiol levels. Confirmatory univariate mixed-effect models established the strength and direction of effects.
Analyses showed that oestradiol levels were primarily related to brain structure. Implicated regions included the ventrolateral pre-frontal cortex, superior temporal sulcus, precuneus and ventral anterior cingulate. White matter microstructure of frontal-temporal connections was also implicated.

Conclusion. Analyses suggested that the structure of brain regions underlying social and emotional processes were associated with oestradiol levels in 9-12 year-old female adolescents. Findings might reflect an organisational role of oestradiol on the developing brain.

abs #091

Living Experience Perspectives to Enhance Suicide Prevention

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Background. It is imperative to acknowledge that within each statistic of suicide-related deaths lies an individual, each possessing a distinct narrative and lived experience. Despite the increasing emphasis on interventions and preventative strategies tailored to specific methods, there remains a paucity of research exploring the narratives and experiences of individuals with lived and living experience. While epidemiological studies gain interest, a comprehensive understanding of the factors associated with the method-specific risk of hanging requires an exploration of lived and living experience. This first-person experience includes the perspectives held by both survivors of hanging attempts and those who have extended support to others. By providing a platform to share these narratives, we can obtain valuable insights into the circumstances, decision-making processes, reasoning, and impulsivity behind the choice of this method, thus contributing to more effective prevention measures.

Methods/Results. This paper presents preliminary findings from a series of semi-structured interviews. These interviews were conducted with four female participants. Their ages range between 27 and 47 years, with an average of 34 years. Two participants discuss their personal experiences of surviving suicide attempts involving hanging, with one of them additionally providing support to a loved one following their own hanging suicide attempt. The remaining two participants have provided support to a family member who had previously attempted suicide by hanging.

Conclusion. Emergent themes from these interviews are discussed, along with implications for the study moving forward.

abs #094

A systematic review of neurological biomarkers to predict antipsychotic treatment response

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Background. Up to a third of patients with schizophrenia do not experience sufficient improvement of symptoms and functioning on their first two trials of antipsychotic medications. This is concerning as ineffective antipsychotic treatment early in psychotic illness can lead to exacerbations of psychotic symptoms that impair daily functioning – leading to worse long-term outcomes. Given the considerable burdens associated with psychosis, there is a clear need to identify successful treatments, earlier in the course of illness. Numerous efforts have been made to identify different biomarkers for predicting antipsychotic treatment responses for patients with psychotic disorders. However, there are no known published systematic reviews pulling available evidence on this topic together. This systematic review is currently in preparation, and aims to identify any previously studied neurological biomarkers proposed to be potential predictors of antipsychotic treatment response in individuals presenting with First Episode Psychosis (FEP) or a psychotic disorder.

Methods/Results. The study is being conducted in accordance with the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic search of three electronic databases (MEDLINE, PsycINFO, and EMBASE) was conducted in May 2022. Data synthesis is currently in progress.

Results: Relevant data from selected studies has been extracted, and the narrative synthesis is currently underway to consolidate all available research on neurological biomarkers that have potential to predict antipsychotic treatment response in individuals presenting with FEP or a psychotic disorder. The clinical utility of the different neurological biomarkers in the prediction of antipsychotic treatment response will be discussed."

Conclusion. Through identifying the most efficacious treatments as early into a patient's treatment as possible, clinicians can avoid the prescription of ineffective drugs and enable more rapid treatment responses. This can enhance patient welfare, while also bringing a considerable economy to the healthcare system.

abs #109

Predictive Modelling of Suicide Ideation and Behaviours Among Youth Using Machine Learning

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Background. Despite the advanced statistical methods used in suicide prediction modelling, most current models are limited by cross-sectional data or show poor accuracy in predicting future suicide thoughts or behaviours (STB). This study aimed to validate a penalised logistic regression model, previously developed to classify children with STB history from clinical and healthy control groups. We aimed to examine whether this baseline model can predict the new onset of STB within two-year follow-ups.

Methods/Results. The current study included children from three waves of data in the Adolescent Brain Cognitive Development (ABCD) study (n = 5,885, 47.8% female, 9-11 years of age at baseline). The previously developed baseline model was applied in the current study to predict the new onset of STB during any of the two follow-up assessments. Our model distinguished between (1) children with no baseline STB, but STB was present at any of the follow-ups (STB group); (2) children with a mental illness but no baseline STB (clinical control); and (3) healthy control children.

Our model was effective in distinguishing the future STB group from healthy controls (area under the receiver operating characteristics curve: 0.74), but less so in comparison to clinical controls (0.64). The factors that prospectively predicted the STB group included depressive symptoms, prodromal psychosis symptoms, impulsivity, family conflict, and a history of mental health treatment.

Conclusion. Our model, using only baseline predictors, effectively predicted future STB risks in comparison to healthy control. Future research should incorporate time-varying risk factors that may better distinguish between the clinical and STB groups over time.

abs #149

Understanding brain volume heterogeneity through polygenic risk profiling of psychiatric disorders and commonly comorbid medical conditions

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Background. Neuropsychiatric disorders display diverse brain volume reductions even within diagnostic groups. Recent research links weak yet significant genetic risk variants to brain volume. We suggest that variable genetic risk profiles for both psychiatric and non-psychiatric conditions better explain this diversity. Our study examines polygenic risk connections between psychiatric disorders, comorbid chronic conditions, and their effects on brain volume deviations.

Methods/Results. We analysed genotype and MRI data of healthy adults from the UK Biobank (N=7,908, age=56±8, 45% male). Polygenic risk scores (PRSs) were computed for 5 psychiatric traits and 16 chronic medical conditions overrepresented in psychiatric cases. Normative models calculated individual brain volume deviations from median values for age and sex. Linear regressions tested correlations between disorder PRS and associations of each PRS with regional volume deviations. Spatial correspondence tests assessed overlap in trait PRS associations with regional deviation profiles.

Chronic conditions exhibited lower pairwise PRS correlations than psychiatric disorders, suggesting distinct aetiologies. Despite genetic and clinical differences (n=69 disease-pairs), 17% of trait pairs shared brain deviation associations (r range=0.21-0.41; $p<0.05$). Conversely, among genetically correlated disease-pairs (n=141), 23% displayed significant spatial correspondence in brain volume deviation profiles (r range=0.17-0.42; $p<0.05$).

Conclusion. We found chronic diseases with distinct genetic bases mirroring similar brain volume deviation profiles, while shared genetic components in psychiatric disorders resulted in varying profiles, akin to pleiotropy. Moving beyond single genetic trait analyses enhances understanding of complex relationships between genetic architecture and phenotypic brain volume variability. These insights contribute to predictive models for neuro-phenotypic outcomes in psychiatry.

abs #154

MULTIVARIATE BRAIN STRUCTURE-COGNITION SIGNATURES OF EARLY PSYCHOSIS

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Background. Cognitive impairment is one of the major symptoms of recent-onset psychosis (ROP), not improving with medications and affecting functional outcomes. ROP individuals present widespread grey matter (GM) reductions and widespread and subtle white matter (WM) abnormalities, but their association with cognitive impairment remains unclear. Using a novel, multiblock partial least squares correlation (MB-PLS-C) analysis, we examined GM-WM relationships and assessed their relationship with cognitive abilities in ROP individuals. We hypothesised that MB-PLS-C would show differential GM-WM patterns between groups, correlated with disease-specific cognitive abilities.

Methods/Results. Using T1 and diffusion-weighted MRI scans of 71 nonaffective ROP patients (age 22.1±3.2) and 71 healthy controls (age 22.1±3.1) from the dataset of Human Connectome Project for Early psychosis, we performed MB-PLS-C analyses between GM and WM variables to identify GM-WM patterns and analysed their correlations with cognitive abilities.
MB-PLS-C between GM thickness and fractional anisotropy (FA), a measure of white matter integrity, demonstrated HC-specific and ROP-specific patterns, explaining 16.92% and 12.38% of the total covariance. MB-PLS-C between GM surface area and FA exhibited shared and differential patterns between groups, explaining 53.21% and 18.97% covariance. The thickness-FA ROP-specific pattern was associated with processing speed, working memory, and episodic memory, while the surface area-FA differential pattern was related to reading abilities.

Conclusion. MB-PLS-C demonstrated patient-specific and differential GM-WM patterns, indicating a potential signature of brain alterations in early-stage schizophrenia. Furthermore, we identified cognitive abilities significantly related to the signature patterns. GM-WM patterns associated with cognitive impairment indicate the mechanisms underlying the symptom and provide insights into treatment targets.

abs #156

The right to mental health: A policy review of national Indonesian policies around the promotion of mental health and wellbeing in schools

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Background. Physical and mental health are expected outcomes of national education, but the extent to which mental health is facilitated at schools is unclear in national policies. We undertook a review of school mental health policies to identify what frameworks are used and what actions are recommended for schools and governments to facilitate students' rights to mental health at school.

Methods/Results. We searched national public policies from 2003-2023 through ministerial databases, Google, and Google Scholar using combinations of terms in Bahasa Indonesia (peraturan, pedoman, bimbingan, sekolah, sehat, mental, konseling, psikologis). Textual data synthesis was undertaken. Thirty-one documents (19 regulations, 12 guidelines/manuals) met the inclusion criteria. Most national policies mandated the Health-promoting School framework, to be implemented by the school health unit (Usaha Kesehatan Sekolah; UKS). UKS appear primarily focused on physical health, with more recent appreciation of the need to invest more in mental health. Universal and whole-school approaches toward mental health and wellbeing have informed Trias-UKS, which encourages schools to focus on delivering health-education, providing a healthy school-environment, and facilitating support and services, while governments are expected to commit as developers, regulators, facilitators, and evaluators.

Conclusion. Provision of students' rights to mental health at schools requires investments that facilitate actions from schools, governments, and communities.

abs #168

Propping up the bar: A qualitative analysis of stakeholder perspectives on psychosocial workplace hazards in late-night hospitality

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Background. The working conditions and culture that typify hospitality work such as long and anti-social hours, shift-work, insecure work, job strain, job stress, casualization, aggression and bullying are known determinants of poor mental health and the COVID-19 pandemic exacerbated many existing stressors.

Methods/Results. This study conducted a series of semi-structured interviews with staff and owners working in Melbourne late-night bars, pubs and clubs. Incorporating an interpretive phenomenological analytical approach, data were analysed through a codebook method, generating a 'thematic map' of key concerns, protective factors and areas for improvements.

Public health strategies early in the pandemic increased insecure work and financial strain, isolation, anxiety and depression but also allowed staff time to reflect on their personal goals and build new habits. Many staff chose to leave late-night work, increasing job demands and stress for remaining staff. Customer interactions worsened post-lockdowns, reportedly 'completely forgotten how to behave'. Sexual harassment is rife; customers were common perpetrators, but so were colleagues and other industry workers and those incidents were more difficult to manage and report, given the intricate social networks between workplaces. Owners had a significant role in working conditions and workplace culture, however, managers were the linchpin for managing relationships and supporting mental health.

Conclusion. Industry culture can foster camaraderie, networks and social support, however, can also perpetuate poor mental-health behaviours and preclude reports of sexual harassment. Positive interpersonal relationships between management, staff and owners are crucial, and amongst the most-feasible areas for improving and supporting mental health in late-night hospitality workplaces.

Neuroscience

abs #009

Neurofilament light chain and glial fibrillary acidic proteins as a biomarker for mood disorders: A systematic review

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Background. There is growing usage of blood-based biomarkers of neuronal injury (neurofilament light chain; NfL) and neuroinflammation (glial fibrillary acidic protein; GFAP) for diagnosis and prognosis in neurodegenerative conditions including multiple sclerosis. However, current literature is mixed as to whether these biomarkers are mildly elevated in mood and anxiety disorders. This is important to clarify given neurodegenerative diseases can present with psychiatric presentations especially in the prodromal phase. The aim of this systematic review is to identify the association between neuronal and glial biomarkers in patients with mood and anxiety disorders compared to healthy controls. If appropriate, we will perform meta-regression analyses of comparing severity of mood and anxiety disorders with biomarker levels, as well as performing subgroup analyses of those with later-onset illness.

Methods/Results. A protocol for a systematic review has been submitted to PROSPERO, with the initial literature search identify >1,500 papers.

Conclusion. We plan to complete the review and present the findings at MDHS Graduate Research Conference 2023. Our findings will be important for the translation of these biomarkers to clinical practice, as it will either validate the use to differentiate neurodegenerative and psychiatric disorders. Furthermore, we may potentially identify phenotypes of mood and and anxiety disorders where these blood-based biomarkers have prognostic and diagnostic utility.

abs #013

Visual Snow Syndrome symptoms are more common in poor sleepers

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Background. Visual Snow Syndrome (VSS) is characterised by a range of perceptual phenomena including visual snow (VS), persistent flickering noise in the visual field. Although people with VSS often report symptoms worsen with poor sleep, a recent study found no relationship between sleep quality and rating of the degree of VS experienced. We used a comprehensive measure to determine if sleep quality was associated with the perceived severity of VSS.

Methods/Results. 165 participants with no prior knowledge of VSS were screened for relevant perceptual experiences. They also completed the Pittsburgh Sleep Quality Index (PSQI), and those who had ever experienced VS completed the Visual Snow Handicap Inventory (VSHI). Of 146 participants included for analysis, 115 were female (30 male, 1 non-binary), and their mean age was 18.85 years (SD = 1.74). 96 participants had ever experienced VS, of whom 81 participants experienced it at least monthly, and 11 met the criteria for VSS. Mean PSQI scores were elevated at 6.18 (SD = 10.6); and mean VSHI scores were low (8.73, SD = 10.6). Results of linear regression suggest that poor sleep quality is associated with both increased VSHI scores ($p < .001$, R-Squared = 0.123) and total number of VSS symptoms ($p = .002$, R-Squared = 0.057).

Conclusion. Our results indicate that poor subjective sleep quality is associated with experiencing more VSS symptoms, and with increased perceived severity of those symptoms. As this study was cross-sectional, we are investigating whether VSS symptoms fluctuate with changes in sleep quality, and the directionality of this relationship.

abs #017

Hippocampal Protein Trajectories across Mouse Adolescent Development

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Background. Many neuropsychiatric disorders emerge during adolescence, a vulnerable developmental window for brain maturation. However, the normal trajectories of brain protein changes during adolescence are poorly understood. In this study, we aimed to map the trajectories in hippocampal protein expression between early adolescence to young adulthood in mice. The hippocampus is a region critical for cognition and is highly impacted in schizophrenia and other neuropsychiatric disorders.

Methods/Results. We chose select candidate proteins including markers for major cell types, synapses, myelin, immune response, and plasticity-related proteins, which were semi-quantified by western blot analyses. Hippocampal tissue from male and female mice was analysed at postnatal ages 4.5, 6, 8, 12, and 14 weeks, n=6 per sex per timepoint. We found that most protein trajectories exhibit a significant age effect (2-way ANOVA), with significant differences emerging between time points (Tukey's multiple comparisons). Several proteins display significant changes at 8W, including Parvalbumin, MAP2, PSD95, GluN2b, GFAP, Iba1, C3, and MBP. In comparison, NeuN, CNPase, CREB, BDNF, and IL-1b showed no significant age-related changes. We also observed sex-specific differences in several protein trajectories, illustrating the importance of sex in neurodevelopment.

Conclusion. Our results provide a foundation to examine how these trajectories may deviate in animal models for neuropsychiatric disorders, such as our maternal immune activation model of schizophrenia. Moreover, they are the foundation for our current research, investigating how these protein trajectories are affected by juvenile hippocampal-dependant spatial memory training.

abs #018

A novel mouse model of CUX2 developmental and epileptic encephalopathy

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Background. Genetic epilepsy has been associated with variation in ion channel genes, however, these only account for ~ 25% of the total epilepsy genes. Several other non-ion channel genes are also established causes of epilepsy, including those that encode transcription factors. Homeobox 2 protein cut-like (CUX2) is a human transcription factor that is expressed in the brain, where it controls neuronal proliferation, dendritic branching, dendritic spine morphology, and synapse formation within the upper-layer II and III (LII/III) neocortical neurons. Genetic variation in CUX2 has been implicated in several neurodevelopmental disorders, including schizophrenia, Autistic Spectrum Disorder (ASD), and severe genetic epilepsy. Particularly, the CUX2 recurrent de novo missense variant, c.1768G>A (E590K) has been identified in patients with developmental and epileptic encephalopathy (DEE), displaying generalized epilepsy, severe cognitive impairment, and autistic features. We engineered the homolog CUX2 E528K homozygous and heterozygous knock-in mouse to study the disease mechanism underlying CUX2 DEE. Homozygous CUX2 E528K mice recapitulated the phenotypic characteristics of patients with the CUX2 E590K variant including active epileptiform spiking and spike-and-wave discharges on electrocorticograms. Subcutaneous pentylenetetrazol assay showed that homozygous and heterozygous CUX2 E528K mice were more susceptible to a proconvulsant challenge suggesting overall increased neuronal network excitability. Homozygous and heterozygous CUX2 E528K mice also demonstrated abnormal hyperactivity and reduced social dominance. The CUX2 E528K mouse provides us with a strong preclinical model to study the pathological mechanisms underlying seizures and comorbidities in CUX2 DEE.

Methods/Results. Homozygous CUX2 E528K mice recapitulated the phenotypic characteristics of patients with the CUX2 E590K variant including active epileptiform spiking and spike-and-wave discharges on electrocorticograms. Subcutaneous pentylenetetrazol assay showed that homozygous and heterozygous CUX2 E528K mice were more susceptible to a proconvulsant challenge suggesting overall increased neuronal network excitability. Homozygous and heterozygous CUX2 E528K mice also demonstrated abnormal hyperactivity and reduced social dominance.

Conclusion. The CUX2 E528K mouse provides us with a strong preclinical model to study the pathological mechanisms underlying seizures and comorbidities in CUX2 DEE.

abs #032

Goal-Directed Behaviour under Uncertainty in the NLGN3R451C/Y Mouse Model of Autism

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Background. Autism is a neurodevelopmental condition caused by the complex interplay between rare genetic changes and the environment. While the clinical landscape is highly variable, impairment across social and non-social cognition is frequently reported. In the present study, we use mice carrying an autism-associated arginine-to-cysteine mutation (R451C) of the neuroligin-3 (Nlgn3) gene: a synaptic adhesion protein widely expressed throughout the brain.

Methods/Results. Using serial probabilistic reversal learning (PRL), a touchscreen-based task assessing goal-directed decision-making under uncertainty, we initially trained mice to respond to one of two identical stimuli: one of which is rewarded and the other unrewarded (deterministic). After five correct responses, the reward contingencies were reversed and mice were required to flexibly alter their response. Reward probabilities were subsequently altered to increase uncertainty (probabilistic), from 100:0 to 80:20, 70:30, and 60:40. Unlike other studies with mice and the serial PRL task, using extensive iterative training and larger reward deliveries, we successfully trained mice to a high caliber (>45% of possible reversals and >65% accuracy at 80:20) of stable task performance. When the association between a particular response and reward delivery was deterministic, the Nlgn3R451C/Y mice were no different from wildtypes in their degree of accuracy or number of reversals. However, the introduction of uncertainty revealed an impairment in their reversal learning driven by a reduction in their win-stay response strategy.

Conclusion. Akin to real-world interactions, where most behaviours are less deterministically associated with their outcomes, the Nlgn3R451C/Y mutation in mice produces phenotypes under uncertainty comparable to those reported in humans with autism.

abs #046

STROMAL CELL-DERIVED FACTOR-1 (SDF-1) ACTS ON BOTH CXCR4 AND CXCR7 IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) TO REGULATE BLOOD PRESSURE.

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Background. Significant progress has been made in establishing links between neuroinflammation, sympathetic nerve activity (SNA), and essential hypertension, yet the exact mechanisms remain unclear. Evidence suggests that a chemokine, stromal cell-derived factor-1 (SDF-1), can increase blood pressure (BP) and SNA. Increased activation of neurons in the rostral ventrolateral medulla (RVLM), a brainstem region critical for the regulation of both sympathetic tone and blood pressure, is linked to increases in both SNA and BP.

Methods/Results. BP was recorded and analysed after microinjections of SDF-1 (0.5ng/nl or 1ng/nl) into the RVLM of anaesthetised Sprague Dawley rats (SDs). AMD3100 (CXCR4 antagonist) and/or anti-CXCR7 (antibody raised against CXCR7) were injected into the RVLM prior to a 1ng/nl SDF-1 microinjection to show that SDF-1 was acting via its receptors. BP and RSNA were recorded and analysed after microinjection of AMD3100 and anti-CXCR7 into the RVLM of spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (WKYs). Triple-labelled immunohistochemistry was performed on 40µm sections of RVLM of naïve SHRs and WKYs for co-localisation of TH (tyrosine hydroxylase; neurons), CXCR4, and CXCR7. Microinjection of SDF-1 (1ng/nl) elicited a significant increase in systolic BP, via activation of CXCR4 and/or CXCR7 receptors; blocking either receptor attenuated this response. Blocking both CXCR4 and CXCR7 significantly decreased BP in SHRs, but not in normotensive WKYs.

Conclusion. These data suggest that neuroinflammation, in part mediated by the SDF-1 and CXCR4/CXCR7 axis, may contribute to the hypertensive state by increasing SNA via activation of RVLM neurons.

abs #053

The HCN channel inhibitor ZD7288 attenuates the alcohol deprivation effect in alcohol-preferring (iP) rats

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Background. The medial habenula (MHb) is implicated in the modulation of stress, aversion, drug intake, and drug withdrawal behaviour. Within MHb, cholinergic neurons densely express pacemaker hyperpolarization-activated cyclic nucleotide-gated cation channel 4 (HCN 4), which regulates intrinsic firing properties by generating an Ih current. Various preclinical studies showed increased firing frequency of cholinergic neurons in long-term nicotine-exposed mice. However, the involvement of HCN channels in alcohol misuse remains undetermined.

Methods/Results. In male and female alcohol-preferring (iP) rats, we examined the localization of HCN4 channels on MHb cholinergic cells with RNAScope in-situ hybridization. Functional characterization of cholinergic neurons was assessed by patch-clamp of brain slices with bath-applied ZD7288. Using acute intracerebroventricular injection (i.c.v.), we examined the role of HCN channels in the alcohol-deprivation effect. Using RT-qPCR, we are evaluating the expression of HCN channels in the MHb along with muscarinic and nicotinic receptors following long-term voluntary alcohol consumption and abstinence. More than 90% of HCN4-positive cells in the ventral MHb are ChAT (Choline Acetyltransferase) -positive and co-express VGLUT1 (Vesicular Glutamate Transporter-1) and VGLUT2 (Vesicular Glutamate Transporter-2). Consistent with these data, bath application of ZD7288 significantly reduced the firing frequency of MHb cholinergic neurons. Acute i.c.v injection of ZD7288 (3mg/5µl) significantly attenuated the alcohol deprivation effect following 14 days of forced abstinence but had no effect on spontaneous locomotor activity.

Conclusion. Our data highlighted the role of HCN channels in the alcohol deprivation effect, current studies are examining if this action involves the MHb and related circuitry.

abs #066

Altered task-related functional connectivity of the frontocingulate cortex in depression

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Background. Activity suppression of the frontocingulate cortex during externally directed attentional tasks reflects progressive disengagement of self-related mental processes. This suppression effect is an adaptive feature of brain function that optimises external goal-directed behaviour. Abnormal activity and functional connectivity of the frontocingulate cortex is consistently observed in depression. However, less is known about the nature of dysfunctional neural patterns during task-related suppression of the frontocingulate cortex.

Methods/Results. The aim of this study was to investigate task-related coupling and decoupling of brain regions with the frontocingulate cortex during task performance in people with depression. Eighty-one 15- to 25-year-olds who met criteria for major depressive disorder (MDD) and 94 matched healthy controls completed a functional magnetic resonance imaging (fMRI) emotional face-matching task. Using psychophysiological (PPI) functional connectivity analysis, we found the groups showed differential connectivity patterns during task performance. Specifically, the MDD group, compared to controls, showed significantly less decoupling of the frontocingulate cortex with task-dependent and cognitive control regions as well as increased coupling with regions implicated in interoceptive processes during the task condition

Conclusion. These results suggest that people with depression may find it difficult to disengage from self-related processes during task engagement and switch their cognitive resources to attend to the task at hand. Our findings help elucidate the neural mechanisms that underlie cognitive and affective disturbances in depression and have important clinical utility as they provide a novel way for identifying individuals who may be experiencing more entrenched, difficult-to-treat depression.

abs #071

Orexin receptor 1 signaling mediates stress-induced binge eating in female mice

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Background. Stress and negative affect are known to trigger overeating, particularly in women. This "emotional eating" is associated with binge eating and higher risk of obesity. The neural mechanisms that underpin this form of dysregulated eating are unclear. The lateral hypothalamic neuropeptides orexin, melanin-concentrating hormone (MCH) and cocaine- and amphetamine- regulated transcript (CART) have been previously implicated in reward, stress and feeding. Thus, we aim to investigate the role of these neuropeptides in stress-induced binge eating in female mice. We hypothesised that lateral hypothalamic neurons expressing the neuropeptides would be significantly activated as a result of stress-induced binge eating as compared to control and that systemic blockade of the respective receptors will ameliorate this behaviour.

Methods/Results. To induce binge eating, mice were subjected to a protocol that employed a mild psychological stressor and intermittent access to highly palatable food. Frustrated mice displayed binge-like behaviour compared to control mice. Brain slices from the lateral hypothalamic area were processed for Fos, orexin, MCH and CART immunostaining. We found significant activation of orexin, but not MCH and CART neurons in the lateral hypothalamus as a result of stress-induced binge eating. Further, mice were acutely administered either the orexin receptor 1 antagonist SB-334867 (15 mg/kg, s.c.) or vehicle (5% DMSO in saline) on test day. Stressed mice treated with vehicle consumed significantly more of the food reward than control mice while stressed mice treated with SB-334867 did not binge.

Conclusion. These data suggest a role for orexin signalling at the orexin receptor 1 in stress-induced binge eating.

abs #079

Effects of intracerebroventricular injection of a miR-124-3p mimic on c-Fos induction in the hypothalamus of wild type mice.

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Background. MicroRNAs are short non-coding RNA sequences involved in post-transcriptional regulation of gene expression, and have shown an effect in depressive and anxious pathogenesis. MiR-124-3p has an established role in depressive and anxious behaviour, dysregulated in human circulating fluids, targeting BDNF and altering behaviour in rodent studies. In prior experiments in our laboratory, intracerebroventricular injection of mimic miR-124-3p altered relevant treatment group behaviour, reducing latency to float and immobility time in the Porsolt test (n=20), and reducing time spent in light in the Light-Dark Box test 24 hours post treatment (n=39). Immunostaining of c-Fos, as a marker of neuronal activity, will show whether these acute modulatory effects on depressive and anxiety-like behaviours are translated molecularly.

Methods/Results. Perfused mouse brains were coronally cut in 30µm slices and stained with anti-c-Fos ab190289 and anti-rabbit Green Alexa 488 between -0.5 to -1.5 from the bregma. Brains were imaged with Leica Thunder imaging as 20x/0.8x dry TileScans. Images of 5-8 slices were cropped to the hypothalamus, and arcuate nucleus, and counted with the experimenter blinded to condition. Counting was performed with Fiji auto-threshold and counting, including cells of 5µm or larger, with a circularity of minimum 0.55.

Conclusion. Mice exposed to the Porsolt test (n=20) exhibited significantly higher c-Fos cell counts to non-exposed mice (n=18). No difference was found between treatment and mimic groups, however treatment non Porsolt-exposed mice (p=0.0114) showed stronger reduction in cell counts to control non Porsolt-exposed (p=0.0335) when compared to control exposed mice.

abs #086

Gastrointestinal dysfunction in transgenic Parkinson's disease mice is associated with altered calcium signalling in the colon

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Background. Gastrointestinal (GI) symptoms in Parkinson's disease often occur years before motor deficits manifest, however, the underlying mechanisms remain elusive and effective therapeutics are yet to be developed. We used a transgenic mouse model that expresses the mutant human A53T alpha-synuclein (α-syn) protein under the direction of the mouse prion protein promoter to investigate them.

Methods/Results. Central deficits were assessed using the ledge beam assay and GI dysfunction was assessed using the fecal pellet output and bead expulsion tests between 12-36 weeks of age. Changes at the tissue and cellular levels were assessed using immunohistochemistry and ex vivo calcium imaging. Transgenic mice exhibited changes in GI function as early as 12 weeks of age, producing a significantly higher numbers of facal pellets ($p=.03$), with comparable weight and water content as wildtype. Colonic motility was also significantly delayed in transgenic mice from 20 weeks of age ($p=.042$). In addition to alterations in overall number of myenteric neurons, transient amplitudes of calcium (Ca^{2+}) signalling in the colonic myenteric plexus, measured via short high K^+ depolarisation and stimulation of the nicotinic cholinergic receptor, were significantly higher in young A53T mice at 12 weeks compared to wildtype ($p<.001$; $p=.001$). This response was reversed at 36 weeks.

Conclusion. A53T transgenic mice exhibit early changes at the tissue and cellular level of the GI tract prior to the manifestation of motor deficits. Differential Ca^{2+} signalling responses to physiological stimuli might be linked to deficits in colonic motility. Understanding these pathophysiology mechanisms could unveil novel therapeutic options.

abs #110

Understanding Toll-interacting protein (TOLLIP) as a regulator of STING activity in traumatic brain injury

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Background. Traumatic brain injury (TBI) is a leading cause of injury-induced death and disability globally. Secondary-injury processes that ensue after TBI, such as neuroinflammation, are known to propagate cell stress and neurodegeneration. We previously confirmed the adaptor protein stimulator of interferon genes (STING) and downstream type-I interferon (IFN) signaling are key modulators of the detrimental neuroinflammatory response after TBI (Abdullah et al., 2018). Toll interacting protein (TOLLIP) has recently been identified as a stabiliser of STING at its resting state (Pokatayev et al., 2020). This study aims to evaluate the role TOLLIP has in regulating STING activity in the CNS post-TBI.

Methods/Results. 10–12-week-old male C57Bl/6 mice were exposed to brain injury using the controlled-cortical impact model (CCI). Mice were killed 2h-and 24h-post TBI and the ipsilateral and contralateral cortex and striatum isolated. Western blot analysis revealed a significant attenuation in the expression of TOLLIP in the cortex 24h-post TBI and not 2h-post TBI when compared to sham. STING protein expression was also found to be decreased, with significant attenuation observed in both the cortex and the striatum 24h-post TBI and no significant alterations 2h-post TBI.

Conclusion. These data suggest that TOLLIP may have a role in modulating the STING-mediated inflammation observed post-TBI. This study aims to address the gap in understanding of how the CNS's innate neuroinflammatory response becomes dysregulated and neurotoxic through the cGAS-STING pathway, and to elucidate if the interplay between TOLLIP, ER-stress and STING observed in the periphery exists in the CNS.

abs #121

Ablation of interferon regulatory factor 7 confers protection in a mouse model of mild traumatic brain injury.

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Background. Traumatic brain injury (TBI) is a major cause of mortality and morbidity worldwide. We have previously confirmed the type-I interferon signaling cascade as a key mediator of the detrimental neuro-inflammatory response in TBI (Karve et al., 2016). To further understand the role of the type-I IFNs in TBI, this study focused on a key modulator of their response, Interferon regulatory factor 7 (IRF7).

Methods/Results. C57BL/6 wildtype (WT) and IRF7-/- male mice (8-10-week-old) were subjected to a controlled-cortical impact (CCI) mouse model of mild TBI (mTBI) (n=7-9 per group). IRF7 mRNA expression was significantly up-regulated in WT mice at 24hr- and 7-day-post injury compared to sham controls (24hr: 3.04±0.41-fold vs 1.04±0.11-fold, p=0.014; 7-day: 11.06±2.02-fold vs 1.07±0.09-fold, p= 0.002, respectively, student's t-test). T2 and diffusion-weighted magnetic resonance imaging analyses of mTBI brains at 7-day-post injury, revealed a statistically significant reduction in cortical lesion size in IRF7-/- mice compared to injured WT mice (2.39±0.46mm3 vs 3.68±0.26mm3, respectively, p=0.026, student's t-test). DigiGait™ analysis aligned with the temporal profiles of lesion sizes, with pre:post injury ratios in ataxia coefficient and stride frequency increased in WT mice 7-days-post injury when compared to sham counterparts (1.09±0.14 vs 1.62±0.16, p=0.011 and 1.08±0.05 vs 1.23±0.037, p=0.018, respectively, two-way ANOVA), with no changes observed in IRF7-/- mice.

Conclusion. This study provides strong evidence for the involvement of IRF7 in regulating the detrimental neuroinflammation following brain injury. Current studies are focused on understanding IRF7's specific role in regulating microglial function and its contribution to the neuro-inflammatory response and secondary damage in TBI.

abs #141

Hypothalamic reactive astrogliosis drives neurofibrosis and metabolic dysfunction.

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Background. Defective and augmented extracellular matrix, a process termed neurofibrosis, develops around neurons within the arcuate nucleus of the hypothalamus (ARC) during the progression of metabolic diseases. Once established, the defective neurofibrotic extracellular environment within the ARC promotes cellular insulin resistance resulting in significant whole-body metabolic dysfunction. Whilst the causality of ARC neurofibrosis to the development of metabolic disease has recently been established, the underlying cellular and molecular mechanisms driving neurofibrosis remain entirely unexplored.

Methods/Results. Key proteoglycan and glycosaminoglycan components of neurofibrosis are expressed by astrocytes, indicating that neurofibrosis may originate through reactive changes in ARC astrocytes. We demonstrated that reactive astrogliosis within the ARC emerges following one week of exposure to a high-fat, high-sugar diet. The degree of astrogliosis in the ARC positively correlate with the development of neurofibrosis within the ARC from 4 weeks and up to 12 weeks of high-fat, high-sugar feeding. To causally link the activity of astrocytes with the development of neurofibrosis, we utilised DREADDs to chemogenetically activate ARC astrocytes in lean chow-fed mice for 8 weeks. We observed a marked development of neurofibrosis within the ARC which drove defective glucose homeostasis. Notably, the effects of ARC astrocyte activation on whole-body metabolism are attenuated following ARC extracellular matrix digestion. In contrast, chemogenetic inhibition of ARC astrocytes in diet-induced obese mice results in the reversal of neurofibrosis, which promotes remission from diet-induced obesity.

Conclusion. These findings define a previously unidentified mechanism by which reactive astrogliosis in the ARC drives the development of metabolic disease through the pathogenesis of neurofibrosis.

abs #163

Characterising regional changes in autophagy in the mouse retina following eye pressure elevation

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Background. Glaucoma is the leading cause of irreversible blindness worldwide and is associated with high eye pressure (or intraocular pressure, IOP). Besides high IOP, ageing is the most important risk factor, however its role in causing glaucomatous retinal ganglion cell loss is still incompletely understood. Recent studies have shown a link between autophagy, a critical cell recycling pathway, and glaucoma. However, age-related changes to autophagy in the retina have yet to be fully explored. In this study, we characterise regional changes in retinal autophagy expression in vivo and compare regional autophagic responses to retinal structure in young and old eyes following IOP elevation using a novel dual-tagged CAG-RFP-EGFP-LC3 reporter mouse model.

Methods/Results. We induced acute IOP (50 mmHg for 30 minutes) via anterior eye cannulation in 3-month-old (n=7) and 12-month-old (n=5) CAG-RFP-EGFP-LC3 mice. At 3 days following injury, we assessed retinal thickness using optical coherence tomography and spatial distribution of intrinsic autophagic fluorescence using in vivo autofluorescence imaging. Compared to younger eyes, older eyes showed significant thickening of the retinal nerve fibre layer ($p=0.037$), but not other retinal layers 3 days after IOP injury. Spatial analysis revealed that these regions of retinal swelling were associated with brighter in vivo fluorescence, indicating reduced autophagy in those areas. Furthermore, older eyes showed steeper slopes when autofluorescence was correlated with total retinal thickness.

Conclusion. These data suggest that in older eyes, regional deficits in autophagy are associated with retinal swelling after IOP injury. However, increased sample sizes are required for further analysis of age-related changes.

Late-Breaking Poster Abstracts

Late-Breaking Poster Abstract

abs #200

General Practice Patients' Experiences of an Approach to Antidepressant Deprescribing: A Mixed-Methods Case Study Realist Evaluation

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Background. Potentially 1 in 3 general practice antidepressant users experience no clinical benefits from their medication and could commence deprescribing. However general practitioners and patients face multiple barriers to deprescribing, and it is not routinely occurring in clinical practice. Patients have been shown to attempt deprescribing without clinician support which can increase the risk of relapse or return to medication. To support safe and successful antidepressant deprescribing in primary care, evidence-based support that includes an understanding of patient experiences is needed.

Aim. To investigate how an existing approach to antidepressant deprescribing works, for whom, and in what contexts by (1) examining the experiences of patients when using the WiserAD structured online support tool for antidepressant deprescribing in general practice, (2) identifying the underlying mechanisms of the WiserAD approach to antidepressant deprescribing, and (3) describing in what contexts and to what extent the underlying mechanisms of WiserAD are suited for antidepressant users in primary care.

Methods. A mixed-methods case study with realist evaluation was nested within the first three months of participation in the WiserAD trial. Narrative style interviews with thirteen WiserAD participants were conducted between October 2022 and April 2023. Qualitative data was coded as "context", "mechanism" and "outcome" (i.e. stage in the deprescribing process) in NVivo. Quantitative data from baseline and 3-month follow-up online surveys was used to characterize participants and identify additional mechanisms.

Findings. Preliminary results show that identifying appropriate patients and initiation of depression, education about antidepressants and deprescribing, guidance from WiserAD and/or a GP to taper, relational support and technical support in the form of daily tracker act as drivers to successful deprescribing.

Implications. This is the first realist evaluation of an antidepressant deprescribing intervention in general practice. Findings from this evaluation may assist in the implementation of antidepressant deprescribing into routine clinical practice.

Late-Breaking Poster Abstract

abs #201

Efficacy and Safety of corneal cross-linking in Keratoconus patients with corneal thickness less than 400 µm

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Purpose. This systematic review analysed the efficacy and safety of corneal collagen crosslinking (CXL) in patients with keratoconus and corneal thickness less than 400µm after epithelium removal.

Background. Keratoconus (KC) is the most common primary ectasia, a bilateral and asymmetric corneal disease. KC is defined by the progressive degeneration and corneal remodelling which mostly affects corneal epithelium and stroma. The main pathophysiology of KC is a disruption in collagen integration in the cornea. CXL is a treatment that uses UVA light and riboflavin to strengthen the corneal collagen fibers and improve the physical characteristics of the cornea. Riboflavin is a photosensitizer and plays its role by creating reactive oxygen species (ROS) to induce collagen cross-links, also as a protective barrier against the harmful influence of UVA. The safety threshold for CXL is a minimum corneal thickness of 400 µm after epithelium removal to have an effective treatment in the anterior corneal stroma and meantime avoid the endothelial layer damage. Due to the progressive nature of KC and thinner cornea in advanced stages, some of the patients are not a good candidate for CXL in traditional methods. Therefore, this increases the chance of corneal transplant as the final treatment for this condition.

Methods. The study followed the PRISMA framework and included studies published in peerreviewed journals in the English language. The search strategy was conducted in MEDLINE Ovid, Embase Ovid, and SCOPUS databases. The study included 20 investigations conducted between 2011 and 2023, and the portion of 2021 was more than others. The geographical distribution of the English records included countries such as Turkey, Italy, Brazil, and Japan. The review conducted meta-analyses for various variables, such as corneal thickness (CCT), thinnest corneal thickness (TCT), and best-corrected visual acuity (BCVA). The quality of the included studies was assessed using the ROBINS-I tool and the JBI Critical Appraisal Checklist for Case Series.

Results. The results showed significant differences in CCT and TCT between the experimental and control groups, while the difference in BCVA was not significant. The heterogeneity between studies was high for CCT and TCT, but there was no heterogeneity for BCVA. The quality of the included studies was assessed using the ROBINS-I tool and the JBI Critical Appraisal Checklist for Case Series. The results indicated there is no heterogeneity between the studies for most variables analysed. These findings suggest that CXL surgery has a significant impact on corneal thickness and cylinder measurements, but its effect on BCVA may not be statistically significant.

Conclusion. Evidence on the characteristics of patients with ultrathin corneas who can safely undergo CXL would improve confidence when making clinical decisions and balancing the risks of adverse events against the chance of progression and potential future need for corneal keratoplasty in advanced keratoconus.