TENOVUS SCOTLAND
SUPPORTING MEDICAL RESEARCH

# TENOVUS SCOTLAND
## Research Symposium

**@TenovusScotland**
**#TenovusSymposium23**

## Friday 6 October 2023

Royal College of Physicians of Edinburgh
11 Queen Street
Edinburgh
EH2 1JQ

Today's Research - Tomorrow's Health

# PROGRAMME

**09.00 – 10.00** **Registration** + tea/coffee/posters

**10.00 – 10.10** **Welcome** and 'housekeeping'
**Professor Ken Paterson,** *National Chair, Tenovus Scotland*

## ORAL PRESENTATIONS

### Session 1: Chair - Professor Jamie Grieve

**Neurology**

**10.10 - 10.25** Spinal cholinergic modulation of breathing in physiological conditions and post-spinal cord injury
*Giulia Benedetta Calabrese, School of Psychology & Neuroscience, University of St Andrews*

**10.25 – 10.40** Novel mouse model of Parkinson's Disease using patient-specific α-synuclein extracts: molecular and behavioural characterisation
*Inga Schmidt, University of Aberdeen*

**Therapeutics**

**10.40 – 10.55** Isoform-selective EPAC activators: Therapeutic opportunities for cardiopulmonary inflammation
*Graeme Barker, Institute of Chemical Sciences, Heriot-Watt University, Edinburgh*

**10.55 – 11.10** Cryoprecipitate transfusion in trauma patients attenuates hyperfibrinolysis and restores normal clot structure and strength; results from a sub-study of the FEISTY trial
*Gael Morrow, School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen*

**11.10 – 11.25** GPR75, a G protein-coupled receptor target for metabolic syndrome treatment
*Fiona Murray, Institute of Medical Sciences, University of Aberdeen*

**11.25 – 11.40** The impact of intensive lipid management on cardiovascular risk after ischaemic stroke - modelling analysis of real-world data
*Cameron Smith, Institute of Cardiovascular & Medical Sciences, University of Glasgow*

**11.40 – 12.00 Tea/coffee/posters**

**12.00 – 12.45 Keynote address: Health care in the 4th industrial revolution**
**Professor Andrew Morris,** *Director, Health Data Research UK*

**12.45 – 13.45 Lunch/posters**

# PROGRAMME

### Session 2: Chair - Professor Alan Foulis

**Oncology**

**13.45 – 14.00** Applying quantitative proteomics to identify plasma proteins associated with oesophageal cancer chemotherapeutic treatment outcomes
*Hasnain Ahmed, School of Medicine, University of St Andrews*

**14.00 – 14.15** Comparing the metabolic signature of breast cancer cells in response to olaparib and capecitabine treatment
*Layla AlNoumas, Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde*

**14.15 – 14.30** Compartment-specific multiomic profiling of ovarian carcinosarcoma identifies candidate drivers of epithelial to mesenchymal transition
*Robert Hollis, The Nicola Murray Centre for Ovarian Cancer, University of Edinburgh*

**14.30 – 14.45** FGF21 reverses HCC associated gene expression in hepatic organoids and HepG2 cells
*Fiona Clegg, Institute of Medical Sciences, University of Aberdeen*

**14.45 – 15.00** Protective functions of p53 in the liver: Investigating molecular mechanisms that limit diet-induced liver disease and a potential biomarker for disease progression
*Celine Wittke, Glasgow Caledonian University*

**15.00 – 15.25 Tea/coffee/posters**

**15.25 – 15.40** **What makes a good submission for Tenovus funding?**
**Professor Susan Pyne,** *Chair of Tenovus Scotland National Scientific Advisory Committee*

### Session 3: Chair - Professor John Connell

**Bio-engineering**

**15.40 – 15.55** Novel polymeric heart valve for global heart valve disease
*Monica Kerr, Department of Biomedical Engineering, University of Strathclyde*

**15.55 – 16.10** Bio-assembly approaches for creating *in-vitro* models of the bone-tendon enthesis
*Vinothini Prabhakaran, Biomedical Sciences, University of Edinburgh*

**Life events**

**16.10 – 16.25** Effective connectivity in chronic pain and adverse childhood experiences
*Georgia Antoniou, Division of Population Health & Genomics, Medical Research Institute, University of Dundee*

**16.25 – 16.40** Multiple and multi-dimensional transitions of 2020 healthcare graduates and the impact of COVID-19
*Lisi Gordon, School of Medicine, University of Dundee*

**16.40 – 17.00 Meeting close and Launch of 'Friends of Tenovus Scotland'**

**17.00 – 19.00 Refreshments and canapés**

## POSTER PRESENTATIONS

**1** Investigating the growth dynamics of multispecies biofilm on medical grade silicone by mesoscopy
*Katherine Baxter, Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde*

**2** A study of altered B cell responses to PAMP-activation in schizophrenia
*Nicole Brace, Division of Biomedical Sciences, University of the Highlands & Islands*

**3** Development and immunogenicity of carbohydrate-based vaccine against Group A streptococcus
*Sowmya Castro, Division of Molecular Microbiology, School of Life Sciences, University of Dundee*

**4** Development of an electrochemical cortisol aptasensor and automated plug-and-play sensor preparation strategy based on consumer-grade materials and processes
*Alexandra Dobrea, Biomedical Engineering Department, University of Strathclyde*

**5** Secondary prevention of colorectal liver metastasis (CRLM) via manipulation of the gut-liver axis
*John Falconer, CRUK Beatson Institute, University of Glasgow*

**6** The neural and behavioural dynamics of apathy in Parkinson's disease: A combined computational and fMRI approach
*William Gilmour, School of Medicine, University of Dundee*

**7** Investigating the role of multiple regulatory domains in aminopeptidases to develop 'living' antibiotics
*Chris Harding, School of Biology, University of St Andrews*

**8** Development and validation of a model to identify people who are unlikely to have atrial fibrillation after stroke
*Giorgios Katsas, School of Cardiovascular & Metabolic Health, University of Glasgow*

**9** The impact of age on the performance of atrial fibrillation risk scores after ischemic stroke or transient ischemic attack
*Giorgios Katsas, School of Cardiovascular & Metabolic Health, University of Glasgow*

**10** Shining a light on brain cancer: Utilising photodynamic therapy in the treatment of glioblastoma
*Danial Kordbacheh, School of Medicine, University of Dundee*

**11** Mechanism of autoantibodies in schizophrenia
*Ryan McLean, Division of Biomedical Research, University of the Highlands & Islands*

**12** The impact of (renal and cardiac) parenchymal cell senescence on macrophage phenotype and phagocytosis
*Katie Mylonas, Centre for Inflammation Research, Institute for Regeneration & Repair, University of Edinburgh*

**13** Investigating the role of mitochondrial transfer in monocyte-derived macrophage driven therapy resistance in acute myeloid leukamia
*Ebubechukwu Nwarunma, Charles Oakley Laboratories, Department of Biological & Biomedical Sciences, Glasgow Caledonian University*

## POSTER PRESENTATIONS

**14** Efficacy of drug-like small molecule analogues of the anti-inflammatory parasitic worm product, ES-62, in protecting against stroke
*Cristina Lumbreras Perales, Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde*

**15** Importance of lipid supplementation for mosquito transmission of human malaria parasite *Plasmodium falciparum*
*Sabyasachi Pradhan, School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow*

**16** Anti-Mullerian hormone but not rapamycin attenuates both cyclophosphamide-induced damage and PI3K signalling activation in human ovarian cortex
*Roseanne Rosario, Biomedical Sciences, University of Edinburgh*

**17** IL-33 in intestinal mucosal immunity
*Molly Scott, Division of Molecular & Clinical Medicine, University of Dundee*

**18** Discovery of DYR726, a brain-penetrant selective inhibitor of PI3K, PDGFR, and the WNT pathway
*Vasudha Tandon, School of Medicine, University of Dundee*

**19** Development of novel polymer-drug conjugates: Towards a multi-target treatment for Alzheimer's disease
*Colin Thompson, School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen*

**20** Alterations in GPCR regulation after high fat diet feeding
*Dawn Thompson, Aberdeen Cardiovascular & Diabetes Centre, Institute of Medical Sciences, University of Aberdeen*

**21** Pioglitazone modelling as a secondary prevention strategy after ischaemic stroke
*Struan Wallis, Institute of Cardiovascular & Medical Sciences, University of Glasgow*

**22** The impact of a Western diet on the cardiovascular system of a transgenic mouse model expressing the single-nucleotide polymorphism (SNP), rs713041, in the human selenoprotein glutathione peroxidase 4 3' untranslated region
*Sarah Walsh, Centre for Obesity Research & Education, School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen*

**23** Towards rapid detection of microplastic particles in human blood samples
*Andrew Ward, Department of Civil & Environmental Engineering, University of Strathclyde*

**24** Investigate retinal cholesterol homeostasis in an Alzheimer's disease mouse model
*Aileen Wong, School of Health & Life Sciences, Glasgow Caledonian University*

**25** Lung stromal cell dynamics are altered by infection experience and ongoing antigen presentation following influenza challenge
*Julie Worrell, Institute of Infection, Immunity & Inflammation, University of Glasgow*

## ORAL PRESENTATIONS

### Spinal cholinergic modulation of breathing in physiological conditions and post-spinal cord injury

*Giulia Benedetta Calabrese[1], Kayla Schardien[2], Matthew Broadhead[1], Anthony Incognito[3], Richard Wilson[3], Michael Lane[2], Simon Sharples[1], Gareth Miles[1]*
*[1]School of Psychology & Neuroscience, University of St Andrews*
*[2]Department of Neurobiology & Anatomy, College of Medicine, Drexel University, Philadelphia, USA*
*[3]Department of Physiology & Pharmacology, University of Calgary, Canada*

Breathing must be readily adjusted to meet changing metabolic demands. It is well established that the rhythm is generated in the brainstem; however, mounting evidence points toward roles for cervical spinal interneurons in adjusting respiratory output. Moreover, propriospinal interneurons are proposed as a key locus for compensatory neuroplasticity following spinal cord injury (SCI). However, the mechanisms are poorly understood.

Here we investigated cholinergic modulation of breathing with a focus on C-boutons – large cholinergic modulatory synapses derived from Pitx2+ interneurons previously shown to facilitate motoneuron output in a task-dependent manner, via M2 muscarinic receptor signalling. Moreover, by making use of a unilateral cervical C2 hemisection injury, we explored the neuroplastic mechanisms by which Pitx2+ interneurons may promote respiratory recovery following SCI.

We found C-bouton synapses on phrenic motoneurons. Pharmacological blockade of spinal M2 receptors reduced the amplitude of respiratory-related activity as recorded from C3/C4 ventral roots in *in-vitro* isolated brainstem-spinal cord preparations from neonatal mice, suggesting that phrenic motoneurons are modulated by endogenous cholinergic signalling. Interestingly, this modulation was reproduced in *in-situ* preparations from adult rats, suggesting that it lasts throughout development. Preliminary results on the SCI model indicate that C-boutons are spared at 4 weeks post-SCI. Ongoing research on a genetic mouse model that lacks C-boutons will provide insights into their contribution to breathing pre- and post-SCI.

Together, these data will offer the first in-depth assessment of the role of Pitx2+ interneurons in the modulation of breathing in physiological conditions and their contribution to the recovery of breathing following SCI.

### Novel mouse model of Parkinson's Disease using patient-specific α-synuclein extracts: molecular and behavioural characterisation

*Inga Schmidt[1], Lianne Robinson[1], Peter Imoesi[1], Amelia Dahlen[1], Maike Müller[1], Stephanie Rommel[2], Bettina Platt[1], Gernot Riedel[1]*
*[1]University of Aberdeen*
*[2]University of Applied Sciences, Kaiserslautern, Germany*

Parkinson's Disease is a neurodegenerative disorder and the advancement towards a cure has stalled for almost two decades. Current murine models of PD try to mimic genetic and environmentally induced forms thereby ignoring the mounting evidence for heterogeneity in the patient population. Recently, a seeding model of PD emerged, using the prion-like properties of α-synuclein, the central toxic protein in PD. Toxic α-synuclein derived from several patients was infused into mice, causing murine α-synuclein to aggregate.

To address the individual differences in patients, we screened a set of PD donor brains and selected four cases, which stood out due to their pathological high versus low α-synuclein and inflammation marker profile. We extracted α-synuclein from these tissues and inoculated individual mouse cohorts with patient-specific extracts. After 6 months, a battery of behavioural tests aiming at general activity, gait and fine motor movements were conducted. In addition, immunohistochemistry and protein quantification of α-synuclein and inflammatory markers was performed.

Our results show differential spread of α-synuclein in all four patient-specific models with inter-patient variation in inflammatory response. Two patient extracts presented with no increase in inflammatory signals. The behavioural analysis revealed a general impairment of fine motor skills in all mice as well as patient-specific differences. This applied to the analysis of gait (CatWalk), but not a smell motivated Buried Cookie test or the Pole descent. This specificity between groups highlights the importance of the selection of the correct tests, but also confirms the heterogeneity of patient extracted α-synuclein species.

This is the first patient-specific mouse model of PD able to mimic subgroups of this heterogenous disease. Using this model for drug testing is a way forward in implementing experimental models of individualised medicine.

### Isoform-selective EPAC activators: Therapeutic opportunities for cardiopulmonary inflammation

*Graeme Barker, Urszula Luchowska-Stańska, David Morgan, Jolanta Weijak, Stephen Yarwood*
*Institute of Chemical Sciences, Heriot-Watt University, Edinburgh*

EPACs are cell-signalling enzymes endogenously activated by cyclic adenosine monophosphate (cAMP) and are known to play a key role in inflammation of cardiovascular and pulmonary endothelia (EPAC1) and insulin exocytosis from pancreatic β-cells (EPAC2). Under normal circumstances, unbound EPACs occupy an autoinhibited conformation; binding of cAMP induces a conformational change which unveils the binding site for Rap, the next species in the signalling cascade, allowing signal transduction (figure 1).

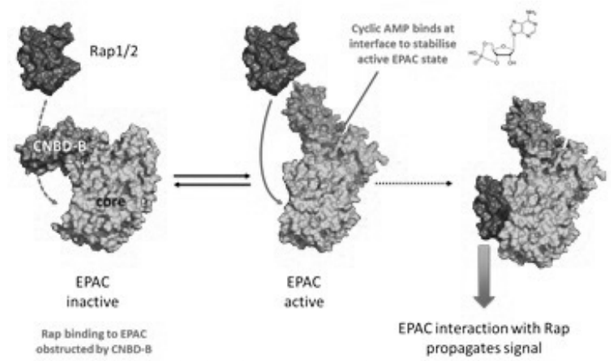


**Figure 1:** EPAC1 activation and signal transduction

Despite their therapeutic relevance, EPACs have not previously been targeted for drug development due to their differing biological roles but identical mode of activation – in addition, cAMP also activates protein kinase A (PKA), cyclic nucleotide-gated ion channels and Popeye domain-containing proteins (POPDCs). Recently, however, we have identified two structurally distinct classes of small molecules which selectively activate EPAC1 without concurrent activation of EPAC2 or PKA (lead compounds shown in figure 2).

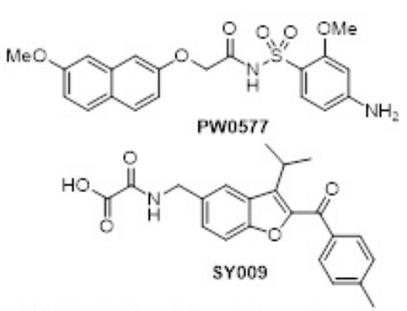


**Figure 2:** Leads from two series of EPAC1-selective activators

We have since conducted ligand-observe NMR studies and generated computational models to explain activation of EPACs by our lead compounds, conducted SAR studies of each series, ascertained EPAC1-selective activation activity in *in-vitro* and in-cell models, as well as determined the effect of our leads in human vascular endothelial cell models in cytotoxicity and protein interaction assays. Recent progress in this area as well as synthesis of lead compounds will be presented as will preliminary results of Tenovus Scotland-funded protein molecular dynamic modelling.

## Cryoprecipitate transfusion in trauma patients attenuates hyperfibrinolysis and restores normal clot structure and strength; results from a sub-study of the FEISTY trial

*Gael Morrow[1,2*], Timea Feller[3], Zoe McQuiltern[4], Elizabeth Wake[5], Robert Ariens[3], James Winearls[5], Nicola Mutch[2], Mike Laffan[6,7], Nicola Curry[1,7]*
*[1]Radcliffe Department of Medicine, University of Oxford*
*[2]Aberdeen Cardiovascular & Diabetes Centre, School of Medicine, Medical Sciences & Nutrition, Institute of Medical Sciences, University of Aberdeen*
*[3]Leeds Thrombosis Collective, Discovery & Translational Science Department, Leeds Institute of Cardiovascular & Metabolic Medicine, University of Leeds*
*[4]Transfusion Research Unit, Monash University, Melbourne & Monash Health, Melbourne, Australia*
*[5]Trauma Service, Gold Coast University Hospital, University of Queensland, Australia*
*[6]Centre for Haematology, Imperial College London*
*[7]Oxford Haemophilia & Thrombosis Centre, NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, Oxford*
**Now at School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen*

**Background** Fibrinogen, the major constituent of a blood clot, rapidly reaches critically low levels during traumatic haemorrhage. Excessive clot breakdown (hyperfibrinolysis) is common and exacerbates low fibrinogen (hypofibrinogenaemia). The Fibrinogen Early in Severe Trauma study (FEISTY;NCT02745041) is the first randomised controlled trial comparing the clinical effects of two different fibrinogen replacement therapies; cryoprecipitate and fibrinogen concentrate (Fg-C); during traumatic haemorrhage.

**Aims** To compare the effect of Fg-C or cryoprecipitate supplementation on clot structure, strength and stability in severely injured patients enrolled to FEISTY.

**Methods** Paired plasma samples pre- and post-fibrinogen replacement were used to investigate fibrin clot structure. The number of fibrin cross-links and clot structure were analysed using confocal microscopy and the mechanical properties of individual fibres investigated using atomic force microscopy (AFM). Healthy donor plasma was used as a control.

**Results** Upon hospital admission trauma patients formed clots with significantly fewer fibrin fibres, that were shorter in length and had reduced cross-links compared to controls. Cryoprecipitate transfusion restored the fibrin network, with fibres comparable to those observed in normal plasma, whereas Fg-C did not restore normal clot structure. AFM analysis confirmed that fibres formed after cryoprecipitate transfusion required more energy to rupture than their Fg-C counterparts.

**Conclusion** In severely injured, bleeding trauma patients, cryoprecipitate supplementation attenuated hyperfibrinolysis, which may be due to the increased PAI-1 and FXIII levels. Patients given cryoprecipitate showed a homogeneous fibrin network with increased fibres than those with Fg-C. Moreover, these fibres showed increased resistance to mechanical stress. Our data indicate that cryoprecipitate may be a superior source of fibrinogen to manage bleeding in trauma coagulopathy by increasing stability against mechanical disruption and fibrinolysis.

## GPR75, a G protein-coupled receptor target for metabolic syndrome treatment

*Cameron Malcolm[1], Jean Iynikel[1], Alasdair Leeson-Payne[2], Anastasios Papadam[1], Sarah Walsh[3], Felix Grassmann[1], Dawn Thompson[1], Lora K Heisler[2], Fiona Murray[1]*
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*[2]The Rowett Institute, University of Aberdeen*
*[3]School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen*

The consumption of a high fat/high sugar diet can result in metabolic syndrome; a group of conditions including insulin resistance, hypertension, and obesity that contribute to heart disease, diabetes, and non-alcoholic fatty liver disease. The exponential rise in these conditions means there is an urgent need for new therapeutics. We and others have found that variants of the orphan G protein coupled receptor, GPR75, are associated with BMI. We have previously found dietary induced obesity to increase GPR75 expression in key organs involved in metabolic syndrome. Using CRISPR/Cas9 technology, we generated GPR75-CRISPR mice to investigate whether the absence of this receptor is protective for the development of high fat diet (HFD)-induced metabolic syndrome. GPR75-CRISPR mice gained significantly less weight on a HFD ((4.5kcal/g, 42.7% carbohydrate, 15.2% protein, 42% fat, ENVIGO, for 12 weeks) compared to WT mice (male: GPR75-CRISPR, 4.8g vs. WT, 15.6g, female: GPR75-CRISPR 1.5g vs. WT 11.2g, n=5-6, $p<0.0001$). Difference in body weight was primarily associated with reduced fat mass (Echo-MRI scanning). In addition, female GPR75-CRISPR mice exhibited improved glucose homeostasis compared to WT controls. QPCR and immunoblotting indicated that GPR75-CRISPR mice had reduced liver fibrosis (>3-fold decrease in Collagen II and αSMA vs. WT liver, n=5-6, $P<0.05$) and lipid storage (>2-fold decrease in PPARγ and Fabp4 vs WT liver, n=5-6, $P<0.05$). Genetic deletion of GPR75 protects against HFD-induced obesity and improved hepatic health. These data validate GPR75 as a novel drug target to treat metabolic syndrome.

## The impact of intensive lipid management on cardiovascular risk after ischaemic stroke - modelling analysis of real-world data

*Cameron Smith, Alan Cameron, Jesse Dawson*
*Institute of Cardiovascular & Medical Sciences, University of Glasgow*

**Introduction** International guidelines recommend an intensive LDL-cholesterol target (<1.8mmol/L) in people with ischaemic stroke or TIA to reduce recurrent cardiovascular (CV) events. We explored the impact of implementing this target using real-world data.

**Methods** We included people admitted with non-cardioembolic ischaemic stroke or TIA within NHS Greater Glasgow and Clyde between 01/12/2015-31/12/2018. We identified people eligible for treatment to an intensive LDL-cholesterol target, defined as not taking maximal therapy and a mean LDL-cholesterol ≥1.8mmol/L in first year post-stroke.

We calculated recurrent rates of recurrent stroke and major CV events (MACE) and estimated the absolute risk reduction (ARR) from the intensive target using treatment effects from the 'Treat Stroke to Target Trial' (HR 0.82 (95%CI:0.63-1.07) for stroke and HR 0.82 (95%CI:0.63-1.07) for MACE).

**Results** We included 4,037 people overall: mean age (+/-SD) 68.6 (+/-12.9) years, 2,053 (50.9%) males. Mean follow-up was 2.19 years. 1,024 were eligible for treatment to the intensive target. Of these, 128 (12.5%) had a recurrent MACE while 120 (11.7%) had a recurrent stroke.

We estimated a change in number of events of -14 (95%CI:-24 to -1) for MACE and -10 (95%CI: -22 to +4) for recurrent stroke. We estimated an ARR of -1.37% (95%CI:-2.34% to -0.10%) (from 12.5%) for MACE and -0.98% (95%CI:-2.15% to +0.39%) (from 11.7%) for recurrent stroke.

**Conclusions** We estimated that implementation of an intensive LDL-cholesterol target (<1.8mmol/L) in a Scottish population would lead to a small but important reduction in number of recurrent CV events in people with ischaemic stroke or TIA.

## Applying quantitative proteomics to identify plasma proteins associated with oesophageal cancer chemotherapeutic treatment outcomes

*Hasnain Ahmed[1,2], Naici Guo[3], Giorgios Minas[3], Silvia Synowsky[2], Margaret Dunne[4,5,6], Rhiannon McShane[1,2], Anshul Bhardwaj[4], Noel Donlon[4,5], Cliona Lorton[4,5,7], Jacintha O'Sullivan[4,5], John Reynolds[4,5], Peter Caie[1], Sally Shirran[2], Andy Lynch[1,3], Alan Stewart[1,2], Swati Arya[1,2]*
*[1] School of Medicine, University of St Andrews*
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*[3] School of Mathematics & Statistics, University of St Andrews*
*[4] Department of Surgery, Trinity Translational Medicine Institute, Trinity College Dublin*
*[5] Trinity St James's Cancer Institute, St James's Hospital, Dublin*
*[6] Department of Applied Science, Technological University Dublin*
*[7] Our Lady's Hospice & Care Services, Harold's Cross, Dublin*

Oesophageal adenocarcinoma (OAC) is a complex and aggressive malignancy which arises from the lower metaplastic transformation of oesophageal mucosa and is associated with inadequate median survival rates. Current treatments only benefit a minority of patients due in part to a lack of differentiation among patient responders and non-responders. Here we utilised quantitative proteomics using Sequential Window Acquisition of all THeoretical fragment-ion spectra-Mass Spectrometry (SWATH-MS) on albumin/IgG-depleted and non-depleted plasma samples taken from 23 patients with locally advanced OAC which were acquired prior to treatment. Patients were stratified based upon their subsequent tumour regression grades (TRG1/2/3 vs TRG4/5) after adjuvant and neo-adjuvant chemotherapy. The depletion of albumin and IgG served to double the number of measurable proteins compared to non-depleted plasma. Using SWATH-MS, we identified substantial differences in the abundance of several proteins between individuals with high and low TRG scores. Proteins of significantly higher abundance in the low TRG group (1-3) included complement C1q subunit proteins, C1QA, C1QB and C1QC. Of those that were found to be of significant abundance in the high TRG group, glutathione S-transferase pi (GSTP1) exhibited the lowest p-value and highest classification accuracy and Cohen's kappa value. The concentrations of these proteins across the groups were verified using ELISA-based assays. This study provides quantitative information relating to differences in the plasma proteome that underpin response to chemotherapeutic treatment in OAC. This work provides a platform for further studies that will aid the development of prognostic assays that can be used to inform OAC treatment regimens.

## Comparing the metabolic signature of breast cancer cells in response to olaparib and capecitabine treatment

*Layla AlNoumas, Nicholas Rattray, Zahra Rattray*
*Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde*

**Background** Worldwide, breast cancer is the most common cancer in females, accounting annually for more than 600,000 deaths and 2.3 million new cases globally. Despite advancements in cancer therapies and accessibility to national breast screening programs, breast cancer remains a major global challenge to diagnose early and treat effectively because of the complex underlying genetic and phenotypic differences in breast tumour subtypes. The triple negative phenotype is the most aggressive subtype due to the absence of hormone receptor expression, including oestrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER-2). The most frequently mutated gene in breast cancer is p53 accounting for 80% of TNBC cases compared to 15% of luminal and HER-2 positive tumours. Mutations in the p53 gene, which is a key DNA repair checkpoint, contribute to the development and progression of breast tumours. DNA repair deficiency in TNBC is associated with genomic instability and metabolic reprogramming, which are hallmarks of cancer. The application of untargeted metabolomics in breast cancer cell drug-dosing studies is a valuable approach to help identify novel pathways associated with molecular sub-phenotypes and treatment-emergent resistance pathways, which has the potential to lead to identify new druggable targets and effective treatment combinations in future.

**Aim** We employed an untargeted metabolomics approach to investigate the metabolome of different breast cancer cell lines, representing aggressive p53-mutated subtypes in response to two anti-cancer drugs: capecitabine, an anti-metabolite and olaparib, a poly ADP ribose polymerase inhibitor, which are both used as standard of care therapies.

**Methods** We exposed a panel of triple negative breast cancer cells to incremental doses of capecitabine and olaparib to measure cell line dependent sensitivity to these agents. Cells were then dosed at the corresponding IC50 dose for a 4–7-day exposure time and analysed by an untargeted metabolomics using a Thermo Exploris 240 coupled to binary Vanquish Ultra High-Performance Liquid Chromatography (UHPLC). Acquired mass spectrometry data were processed in Compound Discoverer 3.3, using principal component analysis (PCA) to visualize and interpret the clustering of quantified metabolite features to examine global differences between treatment and control groups of cell lines examined. Following identification of significantly altered metabolites, we performed pathway enrichment analysis to measure key metabolic changes occurring in response to each treatment using MetaboAnalyst 5.0.

**Results** Our findings showed differential sensitivity of breast cancer cell lines to capecitabine and olaparib treatment that was dose-dependent. Capecitabine treatment in breast cancer cells significantly altered amino acid metabolism, including valine, leucine, and isoleucine, while olaparib predominantly affected fatty acids and sugar phosphates, such as dicarboxylic acids and monosaccharides involved in synthesis and degradation of ketone bodies that play a role in tumour energy metabolism. We plan to validate these findings by matching measurements to our in-house compound library.

**Conclusion** Metabolic profiling helps identify a set of signature metabolites that can potentially be promising in aspects of diagnosis or treatment of aggressive subtypes of breast cancer using targeted analytical approaches for validation. TNBC differential cell sensitivity and mechanisms of treatment-emergent resistance, can be further investigated by probing DNA damage foci accumulation and p53 expression levels, since both olaparib and capecitabine alter DNA synthesis, affecting cancer cell integrity and subsequent mechanism of cell death.

## Compartment-specific multiomic profiling of ovarian carcinosarcoma identifies candidate drivers of epithelial to mesenchymal transition

*Robert Hollis[1], Ailsa Oswald[1], Lorna Stillie[1,2], Ian Croy[1], Michael Churchman[1], C Simon Herrington[1]*
*[1]The Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Scotland Centre, Institute of Genetics & Cancer, University of Edinburgh*
*[2]Cancer Research UK Scotland Centre and Cancer Research UK Beatson Institute, Institute of Cancer Sciences, University of Glasgow*

**Background** Ovarian carcinosarcoma (OCS) is an exceptionally aggressive and understudied ovarian cancer type that harbours distinct carcinomatous and sarcomatous compartments. We now recognise that the sarcomatous compartment forms through complete epithelial to mesenchymal transition (EMT) from carcinomatous cells.

**Aims** To identify shared and compartment-specific events that may represent therapeutically actionable targets, and to identify candidate drivers of sarcomatous compartment formation through EMT.

**Methods** We performed multiomic profiling of matched carcinomatous and sarcomatous components from 12 OCS patients through whole exome sequencing, RNA sequencing and analysis of infiltrating immune cells.

**Results** While paired sarcomatous and carcinomatous compartments demonstrate substantial genomic similarities, multiple loci are recurrently copy number-altered between compartments; regions containing GNAS and SRC are recurrently gained within the sarcomatous compartment.

CCNE1 gain is a common event in OCS, occurring more frequently than in high grade serous ovarian carcinoma (HGSOC) (50% vs 15% in a cohort of 362 HGSOC cases, P=0.004). Transcriptomic analysis shows increased MAPK activity and subtype switching toward poor prognosis HGSOC-derived transcriptomic subtypes within the sarcomatous compartment. The two compartments show global differences in microRNA profiles, with differentially expressed microRNAs targeting EMT-related genes (SIRT1, ZEB2) and regulators of pro-tumourigenic pathways (TGFβ, NOTCH); chrX is a highly enriched target of these microRNAs, and was also frequently deleted across samples. The sarcomatous component harbours significantly fewer CD8-positive cells, suggesting poorer immune engagement.

**Conclusion** CCNE1 gain and chrX loss are frequent events in OCS. SRC gain, increased GNAS expression and microRNA dysregulation represent potential mechanisms driving sarcomatous compartment formation.

## FGF21 reverses HCC associated gene expression in hepatic organoids and HepG2 cells

*Fiona Clegg[1], Dawn Thompson[1], Mairi McLean[2], Graeme Murray[1], Mirela Delibegovic[1]*
*[1]Institute of Medical Sciences, University of Aberdeen*
*[2]School of Medicine, University of Dundee*

Hepatocellular carcinoma (HCC) is a leading cause of cancer related death, with underlying chronic liver disease (CLD) the best recognised risk factor. Fibroblast growth factor 21 (FGF21) is a key metabolic regulator and treatment target for fatty liver disease. Animals lacking FGF21 develop HCC at an accelerated rate. We define the role of FGF21 on *in-vitro* CLD and HCC models.

Adult tissue-residing stem cell derived hepatic organoids were generated from fresh human liver tissue. Immortalised human HCC cell line HepG2 was cultured. Cell models were treated with palmitic acid (PA), lipopolysaccharide (LPS) and tumour necrosis factor α (TNFα) with/without recombinant FGF21 for 24 hours, recapitulating CLD and HCC. Expression of genes associated with HCC were assessed using RT-PCR.

Hepatic organoids had a similar transcriptome to human liver tissue assessed by RNAsequencing. IL-8 expression was significantly increased in both models in response to the PA, LPS and TNFα as expected. Increase in cancer-related gene expression in response to PA, LPS and TNFα was seen in both organoids (Ki67, p53, Claudin 1, HIF1A) and HepG2 (βcatenin and Axin2). Resolution was seen in Ki67, p53, βcatenin and Axin2 with rFGF21.

Our hepatic organoids were validated as suitable models of human hepatocytes. Discrepancies in gene expression changes seen between organoids and HepG2 highlight the importance of differing cell models. The normalisation of gene expression changes seen with FGF21 suggest a potential target both for prevention and treatment of HCC in humans.

## Protective functions of p53 in the liver: investigating molecular mechanisms that limit diet-induced liver disease and a potential biomarker for disease progression

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Liver cancer is a common and lethal disease with few treatment options. As a result of the obesity epidemic, a rising proportion of liver cancer results from individuals first developing a condition called non-alcoholic steatohepatitis (NASH) rather than from Hepatitis infection. NASH is an advanced form of so-called diet-induced (or 'fatty') liver disease. As the name suggests, diet-induced liver disease arises from overconsumption of a fat and sugar-rich 'Western' diet. Rates of liver cancer in Scotland are ~1.5 times higher than average in the UK. Worryingly, obesity and type 2 diabetes - key contributors to developing NASH - are also most common in Scotland. Developing novel treatment or prevention strategies and new methods to diagnose 'at-risk' NASH is critical to combat NASH-derived cancer.

Here, we present evidence that p53, an important defender against cancer, plays a similarly protective role in diet-induced NASH. Our findings suggest that p53 helps to control undue lipid peroxidation, a pro-inflammatory feature of NASH. We also report that a gene called TIGAR, a p53 target and mediator of the anti-oxidant response, is involved in this process. While the loss of TIGAR accelerates diet-induced liver disease, administration of an anti-oxidant halts this process - suggesting a potential intervention. In addition, we report that changes in caffeine metabolism correlate with the development of diet-induced liver disease. This suggests a potential biomarker for NASH. In total, our work provides new insight into molecular mechanisms that protect against NASH and suggests a potential novel biomarker to assess liver disease.

## Novel polymeric heart valve for global heart valve disease

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Cardiovascular disease (CVD) remains the leading cause of global deaths and a major contributor to disability. Whilst in developed countries, cardiac valve disease is an ageing problem, rheumatic heart disease remains the most prevalent cause elsewhere. This includes aortic valve stenosis, affecting 9 million people worldwide and mitral regurgitation, affecting 24 million people worldwide. Valve replacement with a prosthesis remains the most effective treatment against aortic stenosis, presenting a lifesaving procedure. However, existing prosthetic heart valves have limitations which impact their use in low- and middle-income countries, such as the need for costly lifelong anti-coagulant therapy. The Wheatley Heart Valve (WHV) is a novel patented synthetic heart valve designed to overcome the limitations of existing heart valves by facilitating helical flow as blood exists the aorta. This generates a sinus washout of blood as the valve closes, alleviating the risks from thrombosis. This research sets out to validate and optimise the design of this technology through development of a bespoke test system in accordance with standards ISO 5840 parts 1 and 2. Early computational fluid models including a steady state analysis validating our current system, confirmed the unique curvature of the WHV facilitates helical blood flow downstream of the valve. Work is ongoing to optimise our *in-vitro* system to include a full aortic model (including coronary arteries and arch) to visualise this spiral flow and sinus washout phenomenon beyond the aortic root. This work will advance the WHV closer towards clinical translation.

## Bio-assembly approaches for creating in-vitro models of the bone-tendon enthesis

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The bone-tendon enthesis is an anatomically complex structure and is a common site of orthopaedic injury. Attempts to create enthesis structure and function *in-vitro* via tissue-engineering approaches have been varied, yet none have recapitulated the unique structure and function of the interface, limiting orthopaedic repair strategies thus far. To address this, our study aimed to develop a novel model of the enthesis *in-vitro*, by fabricating 3D mini-tissues of bone, tendon and bone marrow stem cells with a customised bio-assembly apparatus. Pillar array supports (0.5 mm pillar-to-pillar distance) were CAD designed, printed using a Formlabs Form2 3D printer and post-processed. Spheroids of osteoblasts (dRObs), fibroblasts (RTFs) and bone marrow stem cells (BMSC)

were generated using 96-well U-bottom cell repellent plates. Spheroids of ~1.5 mm (dRObs: day 15, RTF: day 3; BMSC: day 1) were deposited in between pillar arrays using bio-assembly apparatus and cultured in appropriate media at 37°C and 5% CO2. Spheroid fusion was assessed using H&E staining after removal of spheroids from supports on day 2, 4 and 6. Fused spheroids were further cultured up to 8 days in scaffold-free conditions and histologically assessed. dROb spheroids fused within two days in supports and formed mini-tissues in scaffold-free conditions with calcium deposits visible by alizarin red and von kossa staining. To date, no fusion of RTF and BMSC spheroids has been observed, requiring further investigation. Our future work will assess the ability of spheroid-based mini-tissues to create an enthesis model *in-vitro*.

## Effective connectivity in chronic pain and adverse childhood experiences

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**Background** Adverse childhood experiences (ACEs) may result in persistent changes in behaviour and stress reactivity, associated with a greater risk of mental and physical health issues, such as chronic pain (CP) and mood disorders. We aimed to investigate whether the brain salience network is linked to self-reported ACEs and CP, while also exploring their effective connectivity (EC).

**Methods** The Generation Scotland Scottish Family Health Study (GS:SFHS) dataset, a longitudinal community-based research dataset, was utilised. This contains socio-demographic and clinical data, including the Chronic Pain Grade. We analysed a subset of the GS:SFHS participants, who participated in the Stratifying Resilience and Depression Longitudinally (STRADL) study and completed assessments, including the childhood trauma questionnaire, neuroimaging, and the implicit emotional processing task. Dynamic causal modelling analysis was performed to test hypotheses linking the brain regions of interest and questionnaire data. Regions of interest were the anterior cingulate cortex (ACC), insula and thalamus.

**Results** The dataset included a total of 579 individuals with a mean (SD) age of 58.15 (10.00) years, 349/579 (60%) females. 238 had reported CP, of whom 159 (67%) were females. Higher EC from the ACC to the insula was associated with individuals reporting CP. The EC from the ACC to the thalamus showed an inverse association with both reported CP and emotional abuse, while there was a positive association with both reported CP and emotional neglect.

**Conclusions** The results highlight the involvement of the ACC and insula in neuropathology associated with CP and ACEs.

## Multiple and multi-dimensional transitions of 2020 healthcare graduates and the impact of COVID-19

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**Aim** This study aimed to explore the impact of the COVID-19 pandemic on the transitions of 2020 medicine, nursing and dentistry graduates into clinical practice to understand their ongoing transition support and wellbeing needs.

**Methodology** This was a qualitative longitudinal study, with semi-structured interviews undertaken at two to three time points. A total of 45 interviews were undertaken. Data were collected from healthcare students who were due to graduate in 2020 from University X and were either practising in NHS Y Region or were unable to graduate because of COVID.

**Results** Five main themes emerged.
1 The timing of transitions to professional life was different for the three groups.
2 Their expectations did not meet reality, especially related to roles, support and supervision, and illness and death at such a large scale.
3 They experienced multiple professional *and personal* transitions. Further, their transitions triggered transitions for their families and peers.
4 The uncertainty, COVID related deaths and lack of support heightened their anxiety. However, some highlighted their sense of pride and enhanced learning.
5 Some reported strong support systems at work and home, others reported lack of support at work.

**Conclusion and implications** The study had several implications for policy and practice related to educational supervisors, senior staff, shadowing/interim role, other support systems and formal communication.

This comic captures the study
https://discovery.dundee.ac.uk/ws/portalfiles/portal/74586195/Tenovus_comic_ENG_mobile_22.05.24.pdf

# POSTER PRESENTATIONS

## 1 Investigating the growth dynamics of multispecies biofilm on medical grade silicone by mesoscopy

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Indwelling medical devices can act as a point of entry and growth surfaces for colonisation by commensal organisms carried on the skin. The silicone elastomer polydimethylsiloxane (PDMS) is a component in many of these devices, and is readily colonised by biofilms of the skin microflora *Staphylococcus aureus* and *Candida albicans*. These biofilm infections have greater virulence and resistance to antimicrobial drugs, resulting in poor patient outcome and increased treatment costs

We are using the Mesolens 3D imaging system to visualise large areas of the *C albicans/S aureus* biofilm. By using an objective lens combining high numerical aperture with low magnification, we can image global biofilm structures in great spatial detail. Fluorescently labelled *C albicans* and *S aureus* strains are used to study single and dual species biofilm formation on a mimic of a PDMS indwelling medical device, allowing us to study how biofilm architecture alters in the presence and absence of an agar tissue equivalent. Our results show a greater biomass and differences in biofilm structure on PDMS mounted in agar than on PDMS alone, suggesting adherence and growth to PDMS may be more successful when inserted into a biotic substrate.

Biofilms are a serious threat to human health. The study of their architecture will further our understanding of how biofilms intrinsically promote virulence and antimicrobial resistance, helping us develop new tools against them.

## 2 A study of altered B cell responses to PAMP-activation in schizophrenia

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Schizophrenia is a severe mental health condition though the causes remain largely unclear. Infection is thought to be a risk factor for schizophrenia and a reduced ability to regulate the immune response may contribute to disease pathogenesis. B cells form a crucial component of protective immunity to infection and primarily serve as antigen presenting cells, by presenting self or foreign peptide fragments on major histocompatibility complexes (MHC) to T cells. Antigen-activated T cells will in turn either undergo immune tolerance to self or promote B cell differentiation into memory cells or antibody-producing plasma cells. B cells also express toll-like receptors (TLRs) that can recognise pathogen-associated molecular patterns (PAMP) resulting in distinct immunological events, including cytokine production and the promotion of antigen presentation. Some patients with schizophrenia show an imbalance in the TLR signalling pathways.

This study will monitor PAMP stimulation of B-Lymphoblastoid cell lines (B-LCLs), derived from schizophrenia patients and healthy controls, using the TLR7 agonist imiquimod (R837). The effect on B cell function via proliferation, cytokine production and cell surface expression will be monitored. Furthermore, immunopeptidomics will be conducted to identify changes to the expression of MHC-class II peptides following PAMP stimulation. Ultimately this study may further identify altered B cell responses to immune activation as a contribution to the pathogenesis of schizophrenia.

## 3 Development and immunogenicity of carbohydrate-based vaccine against Group A Streptococcus

*Sowmya Castro, Division of Molecular Microbiology, School of Life Sciences, University of Dundee*

Group A Streptococcus (GAS) also known as *Streptococcus pyogenes* is a Gram positive human exclusive pathogen responsible for causing annually 600 million cases of pharyngitis, commonly referred as strep throat. GAS colonise on the throat and skin causing infection such as tonsillitis and impetigo and serious infectious such as scarlet fever and post streptococcal immune mediated diseases. The prevalence of rheumatic heart disease is one of the greatest burdens in the low- and middle-income countries affecting 15.6 million people each year. The extreme antigenic and genetic diversity found in GAS strain is one of the vital reasons for no commercial vaccine available to date. Group A Carbohydrate (GAC) is one of the major components of GAS making 50% by weight, of the cell wall. GAC is conserved in 100% of all isolated serotypes so far. We have developed GAC as an immunogen against GAS using recombinant technology in *E coli*. The protein glycan coupling technology employed produce a dual-hit vaccine with carrier protein and glycan from different pathogens. Our current work is built on our previously funded project by Tenovus Scotland. Here, we will be discussing the immunogenicity of our glycoconjugate vaccine candidate produced from the immunised mice and rabbit models and test the specificity of the antibodies against the antigen using a wide range of clinical GAS *emm* strains.

## 4 Development of an electrochemical cortisol aptasensor and automated plug-and-play sensor preparation strategy based on consumer-grade materials and processes

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Cortisol is a steroid hormone that has gathered significant attention in the biosensing world due to its myriad physiological roles in health and disease. Because of its circadian rhythmicity and fast responsiveness to common sampling procedures (eg blood collection) and everyday stressors, multiple samples must be collected over the time period of interest (weeks or months) to gain a true understanding of its secretion patterns. However, state-of-the art cortisol quantification techniques such as mass spectroscopy are expensive and time consuming posing a roadblock to performing large-scale, long-term cortisol investigations with sufficiently high temporal resolution in a cost-effective manner. This work presents a proof-of-concept label-free low-cost electrochemical aptasensor based on a duplex dissociation principle, using an aptamer first reported by Yang et al (2017). With a linear range of 625 – 10,000 pg/mL, the limit of detection was 625 pg/mL in spiked buffer; for reference salivary cortisol levels for healthy adults are 10.2–27.3 ng/mL (morning) and 2.2–4.1 ng/mL (night). Although these sensors hold great promise for reducing the costs of biomarker investigations, sensor preparation is often performed manually which increases the risk of sensor variability and lowers reliability. To address this issue, a low-cost fluidic cell was developed using laser-cut acrylic sheets and off-the-shelf stick-and-play tape to allow for precise delivery of reagents to the sensor surface. The flow cell design can be easily modified to fit any planar electrode format and was shown to significantly decrease SAM variability during sensor functionalisation (CoV dropped from 47% to 6%).

## 5 Secondary prevention of colorectal liver metastasis (CRLM) via manipulation of the gut-liver axis

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Obesity and associated high liver fat content are known to increase the spread of metastatic disease in cases of colorectal cancer (CRC). We sought to determine if the administration of a dietary constituent, namely inulin propionate ester (IPE), which reduces weight gain and maintains a lean phenotype, could mitigate this increase and improve metastatic burden in mice.

**Methods and analysis** We performed intrasplenic transplantation in C57BL/6 mice using an aggressive Apcfl/fl, KrasG12D/+, Trp53fl/fl, TrgfbrIfl/fl (AKPT) colorectal cancer organoid line and 4 experimental groups were generated: 60% fat (experimental high fat diet); 60% fat + 10% IPE, 10% fat (normal chow); 10% fat + 10% IPE.

**Results** Experimental mice all gained weight and IPE had a modest effect, generating a leaner phenotype in the setting of high fat diet. There was a trend towards higher metastatic burden in the experimental high fat diet group compared with those fed IPE. No influence of IPE was observed in mice on normal diet. Immunohistochemistry analysis shows that neutrophil numbers are significantly higher in the experimental diet group. No other differences in immune cell populations were observed.

**Conclusion** IPE may protect against weight gain in fatty diets. Liver metastases were reduced by propionate in the high fat diet condition and further studies are required to understand the underlying mechanism.

## 6 The neural and behavioural dynamics of apathy in Parkinson's disease: A combined computational and fMRI approach

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**Objective** To investigate the neural basis of apathy in Parkinson's disease (PD)

**Background** Apathy is a debilitating and poorly understood syndrome characterised by a reduction in goal-directed behaviour. Apathy affects 40% of patients with PD, but its underlying mechanisms are not well understood. Dysfunction within the fronto-striatal circuit the which mediates motivated behaviour has been proposed. This includes the ability to learn, through outcome monitoring, whether a behaviour was worth performing. No previous studies have sought to test whether this explains apathy in PD.

**Method** We examined a cohort of 53 PD patients, 25 of whom were classified as apathetic and 28 as non-apathetic, along with 22 age-matched healthy controls. Participants underwent functional MRI while performing a restless bandit task, a decision-making task that requires monitoring and learning from the outcomes of actions.

We examined both model-free behavioural performance (decision time, missed trials, best bandit choice) and model-based behaviour. Model-based choices were categorised following fitting of behaviour to a reinforcement learning model with two learning rule variants (Temporal difference, Kalman Filter) and four variants of the Softmax choice rule.

**Results** Our findings revealed three core results. Firstly, apathetic patients struggled to choose the best option when faced with outcome uncertainty. Secondly, they employed an exploratory decision strategy, which was best explained by a failure to incorporate the neural representation of an option's value into decision-making. Thirdly, a loss of compensatory neural circuits involved in decision evaluation predicted the manifestation of apathy in PD. Reward insensitivity was identified as a core feature of PD-Apathy, extending previous research. Despite this, our results suggest that this insensitivity to reward is not due to a failure of encoding the value of the chosen option within the brain, but rather a degradation of the neural processes that lead to motivated decisions.

**Conclusion** Our study suggests that apathy in PD arises from a combination of a loss of reward encoding and loss of mechanisms which override decision noise. The increased activation of the explore circuit in non-apathetic PD may represent a compensatory mechanism that protects against the manifestation of apathy. Future research should aim to augment neural circuit compensation and protect from the manifestation of apathy in PD. Targeted neuromodulation of regions within this network could be a treatment intervention worth investigating.

## 7 Investigating the role of multiple regulatory domains in aminopeptidases to develop 'living' antibiotics

*Chris Harding, School of Biology, University of St Andrews*

Bacteria need to source and consume nutrients to grow and survive. Proteins are valuable nutrients, that require hydrolysis by a series of enzymes to release amino acids, which can then be used in other metabolic processes. Aminopeptidases target the removal of the terminal amino acid from peptide fragments. Tight control of their activity is essential to avoid hydrolysis of non-target proteins, leading to cell damage and death. Several predatory bacteria species, which rely on degrading prey proteins to survive, possess a class of large multi-modular secreted aminopeptidases. These enzymes couple three regulatory domains (a PDZ domain, a β-propeller domain and a PA domain) to a catalytic aminopeptidase domain. Individually, these domains are known to regulate protease activity and determine substrate selection. However, the interplay between these domains is unknown. This work uses a combination of structural biology, enzymology and biochemistry techniques to uncover the structure and function of these enzymes, which will provide insights into the complex regulatory mechanism and their role in nutrient turnover. Understanding how these enzymes are regulated is fundamentally important and will aid in aid the development of using predatory bacteria as 'living' antibiotics.

## 8 Development and validation of a model to identify people who are unlikely to have atrial fibrillation after stroke

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*School of Cardiovascular & Metabolic Health, University of Glasgow*

**Introduction** Prolonged cardiac monitoring (PCM) is performed to detect atrial fibrillation (AF) after stroke but resources are limited. We aimed to develop a model to identify people who are unlikely to have AF after stroke and improve allocation of PCM resources.

**Method** We analysed registry data of people with ischaemic stroke or transient ischaemic attack and no known AF from 2015-2018. The cohort was randomly split into derivation (n=3,027) and validation (n=1010) groups. Prediction models with clinical ± electrocardiographic (ECG) predictors of no AF within one-year after stroke were constructed by stepwise multivariate regression. AUROCs were compared by DeLong's test.

**Results** We included 4,037 people (mean [SD] age 69 [12.87] years; 49% female) and 201 (5%) had AF detected. The model with clinical variables included no history of hypertension, no heart failure, no previous ischaemic stroke, a lower deprivation decile, and younger age; and demonstrated good predictive capacity for no AF (AUROC 0.70, 95% CI 0.67-0.73). The model with clinical variables and ECG parameters included younger age, no history of lipid-lowering medication, lower QT interval and higher Q offset. The combined model demonstrated good predictive capacity for no AF (AUROC 0.70, 95% CI 0.66-0.75), retained its performance in validation (AUROC 0.69, 95% CI 0.58-0.80) and was superior to the clinical model in terms of goodness of fit and by DeLong's test (p=0.03).

**Conclusion** A model with clinical and ECG predictors can identify people who are unlikely to have AF after stroke and could help to improve allocation of PCM resources.

## 9 The impact of age on the performance of atrial fibrillation risk scores after ischemic stroke or transient ischemic attack

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**Introduction** Risk prediction models (RPMs) have been developed to identify people who are most likely to have atrial fibrillation (AF) after stroke and who would benefit from prolonged cardiac monitoring (PCM). However, limited data exist concerning their performance in older people. We aimed to assess this.

**Method** We used routinely available data from adults admitted with acute ischemic stroke or TIA and without documented AF to NHS Greater Glasgow & Clyde (2015-18). Seven established AF RPMs (HAVOC, C2HEST, CHADS2, CHA2DS2-VASc, HATCH, MAYO and MR-DASH) were compared by c-indices (Delong method) and calibration ratios. The outcome was incident AF detection in the year after index stroke/TIA.

**Results** Among 4037 participants analysed (median [IQR] age 69 [19] years, 49% female), the AF detection rate was significantly higher in individuals aged ≥75 years (8.5%; n=120/1410) than <75 years (3.1%; n=81/2627; p≤ 0.001). In the combined study sample, c-indices were comparable ranging from 0.63-0.69. When stratified by age, c-indices were lower in people aged ≥75 years (from 0.51 [95% CI, 0.46-0.56] for CHA2DS2-VASc to 0.56 [95% CI, 0.51-0.61] for MR-DASH) than in people aged <75 years (from 0.59 [95% CI, 0.53-0.65] for MR-DASH to 0.64 [95% CI, 0.58-0.70] for MAYO). Discriminative capacity differed by age groups for the HAVOC (p=0.03), CHADS2 (p=0.02), CHA2DS2-VASc (p=0.01), and MAYO (p<0.01) scores; but not for C2HEST, HATCH or MR-DASH.

**Conclusion** RPMs performed less well in terms of discriminative accuracy among people aged ≥75 years. These findings suggest caution is required when using RPMs to select older people for poststroke PCM.

## 10 Shining a light on brain cancer: Utilising photodynamic therapy in the treatment of glioblastoma

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Photo diagnosis is used intraoperatively to identify glioblastoma (GBM) tumour and achieve a greater extent of resection. Patients are given a 'pink drink' of 5-aminolevulinic acid (5ALA), a photosensitiser, which is preferentially taken up by cancer cells and fluoresces under certain light frequencies. It has been shown that the use of photosensitisers and light in this process may cause cell death, encouraging the investigation of photodynamic therapy as a potential treatment for GBM.

While the potential of photodynamic therapy (PDT) as a diagnostic option is evolving, PDT's progress in neuro-oncology remains in its infancy. Although reactive oxygen species (ROS) generation has been proposed to be an important factor contributing to cell death in PDT treatment, limited evidence is available to confirm this. We aim to establish the mechanism of cell death observed in primary GBM 2D and 3D cells upon exposure to light and photosensitiser and confirm the efficacy of PDT therapy using an in vitro cell culture system. Primary patient derived glioma cell lines and 3D stem neurospheres were exposed to 630nm LED laser irradiation with the use of 5-ALA photosensitiser. Cell kill was assessed using microscopy and an MTS cell viability assay quantified by a 96-well Tecan plate reader. Assays using CellROX fluorescent dye were used to investigate reactive oxygen species as a mechanism of cell kill. Results show that cell death was not caused by 5ALA and irradiation alone, however, the combined effect of the two incurred cell death in cell lines tested with evidence of ROS playing a role in the mechanism of cell death.

## 11 Mechanism of autoantibodies in schizophrenia

*Ryan McLean, Division of Biomedical Research, University of the Highlands & Islands*

Schizophrenia is a poorly understood mental health disorder. It is likely that communication between cells in the brain is somehow disrupted in patients with the disease, resulting in the symptoms of schizophrenia. Antibodies, which are proteins that normally help fight infection, that are capable of targeting the brain have long been hypothesised to contribute to the development of Schizophrenia, but it is unclear how. Typically, antibodies recognise and bind to their specific targets and, once bound, they contribute to further activation of the immune system. Sometimes antibodies capable of attacking the body's own cells and tissues develop - autoantibodies. Studies suggest that, in schizophrenia, antibodies bind to their target in the brain and may disrupt the functions of that target. However, less well investigated is the ability for antibodies to contribute to inflammatory processes in the brain. The ability for an antibody to be involved in this way is dependent upon the antibody class. This study established a mouse stem-cell derived model of mixed neuronal cell-types, including neurons, oligodendrocytes, and astrocytes. These mixed populations were examined for differentiation markers and then incubated mouse-derived autoantibodies, of different classes, recognising different targets in order to examine the impact of antibody class and antibody target on a number of schizophrenia-relevant neuronal processes including complement production, calcium signalling and the presence of neurotransmitter receptors.

## 12 The impact of (renal and cardiac) parenchymal cell senescence on macrophage phenotype and phagocytosis

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Senescent cells (SCs) accumulate in organs with age/injury. They are metabolically active, promoting inflammation/fibrosis via release of senescence associated secretory phenotype (SASP) cytokines. We showed that ageing, both natural and premature (radiation exposure), induces renal epithelial senescence, with administration of the senolytic drug ABT-263 prior to ischaemia reperfusion injury protective; reducing tissue atrophy, fibrosis and inflammation, whilst promoting structural integrity/ regeneration.

We hypothesise that SCs compromise renal repair by driving excessive macrophage recruitment and polarisation towards a pro-inflammatory phenotype. Ageing also reduces macrophage clearance of SCs through various mechanisms, which we are investigating. Renal disease is associated with cardiac disease. Our work indicates that kidney injury induces remote cardiac senescence/fibrosis, suggesting crosstalk between organs. Understanding the mechanisms behind macrophage failure to clear SCs in either kidney or heart could lead to new approaches to increase macrophage recognition and phagocytosis, thus reducing SC burden.

**Hypothesis** SCs induce an inflammatory phenotype and phagocytic deficiency in macrophages. Experiments, funded by Tenovus Scotland, are currently underway. We are inducing senescence in human cardiomyocytes *in-vitro*. Investigating the link between cardiac and renal senescence, we will determine whether exposure of cardiomyocytes to renal SC conditioned media (CM) induces a senescent phenotype, and vice versa. Human macrophages are being exposed to renal or cardiac SC (or control) CM. Phenotype is being assessed by bulk RNAsequencing, with a focus on inflammatory/phagocytosis pathways. Similarities in transcriptomic changes caused by either type of SC will be instrumental to finding ways to target SC-clearance in both organs simultaneously.

## 13 Investigating the role of mitochondrial transfer in monocyte-derived macrophage-driven therapy resistance in acute myeloid leukaemia

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**Background** Macrophages (Mφs) are broadly classed as M1-like (anti-tumourigenic) or M2-like (pro-tumourigenic). Acute myeloid leukaemia (AML) patient bone marrow (BM) contains a higher number of M2-like Mφs versus BM of healthy individuals. The ability of M2-like Mφs to drive chemoresistance in AML and the mechanism(s) involved however have not been fully elucidated.

**Aims** We investigated the capacity of M2-like monocyte-derived Mφs (MDMs) to protect AML cells against therapy-induced apoptosis and uncover the mechanism(s) underlying this resistance.

**Methods** Healthy blood donor monocytes were differentiated into M2-like Mφs with M-CSF. On day 10, media was utilised as conditioned media (CM); enriched in MDM secreted factors. AML cell lines and primary AML cells, were cultured alone, in the presence of 50% MDM CM or in co-culture with MDMs and treated with daunorubicin (DNR) or cytarabine (Ara-C) for up to 72h. Apoptosis was assessed by eFluor450/Annexin V staining and mitochondrial transfer was evaluated in CFSE-labelled AML cell lines cultured with MitoTracker Deep Red FM loaded MDMs and flow cytometry.

**Results** MDMs completely abolished DNR and Ara-C-induced kill of AML cells. Intriguingly, 50% MDM CM also promoted KG-1a cell survival in the presence of DNR and in primary cells during culture with DNR and Ara-C. Moreover, MitoTracker studies suggest that mitochondria are transferred from MDMs to the AML cell lines.

**Conclusion** These data reveal the ability of M2-like MDMs to protect AML cells against therapy-induced apoptosis. Studies ongoing in our laboratory are exploring blockade of mitochondrial transfer, as a strategy to overcome macrophage-mediated therapy resistance.

## 14 Efficacy of drug-like small molecule analogues of the anti-inflammatory parasitic worm product, ES-62, in protecting against stroke

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**Background** Mutations in the genes COL4A1 and COL4A2 result in cerebral small vessel disease (SVD), a major cause of stroke with no drugs to prevent it. Prevention of inflammation and BBB breakdown are key treatment targets in SVD.

Drug-like small molecule analogues (SMA 11a and SMA 12b) of ES-62, a parasitic worm product, prevent inflammation and barrier breakdown in peripheral diseases but have never been tested in SVD.

**Hypothesis and aims** ES-62 SMAs 11a&12b prevent inflammation and BBB breakdown in SVD.
Research questions:
1 Do SMAs reduce inflammation *in-vivo* in COL4A2+/- mutant mice, a relevant mouse model for SVD?
2 Do SMAs reverse human brain microvascular endothelial cell (HBMEC) dysfunction *in-vitro* in COL4A mutant cells, a pathological feature of BBB breakdown?

**Methods**
1 Aged (8.5-month-old) male and female COL4A2+/- and wild type (WT) mice were randomly treated with SMAs (1ug) and interleukin-1β (IL-1β, 20ng/kg) or vehicles (n=5/group) for 3 months before blind assessment of inflammation.
2 CRISPR-engineered COL4A HBMECs (G755R, n=3-4; and Intron-25 splice, n=2-3) or WT treated with SMAs (0.5 μg/ml) or IL-1β (10ng/ml) or vehicles underwent *in-vitro* blind assessment of migration, viability, and nitric oxide (NO).

**Results** Preliminary data suggest:
1 SMAs potentially reverse the systemic inflammatory burden *in-vivo* in COL4A2+/- mice, mostly on M-CSF, TNF- α, C5a, IL-1ra, IL-5, IL-6, IL-7 and CM-CSF.
2 SMAs potentially reverse reduced migratory function *in-vitro* in Intron-25 cells.

**Impact** For the first time, we hope to determine the efficacy of parasitic worm-related therapies in ameliorating SVD.

## 15 Importance of lipid supplementation for mosquito transmission of human malaria parasite *Plasmodium falciparum*

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Malaria remains a major cause of illness and death globally. The disease is caused by parasites that are transmitted between people through the bite of an infected *Anopheles* mosquito. *Plasmodium falciparum* is the parasite species responsible for the majority of deaths from the disease. One potential disease control strategy is to block parasite transmission to mosquitoes from human host using transmission blocking vaccines or drugs. Novel transmission blocking candidates are usually tested by the Standard Membrane Feeding assay (SMFA) that measures mosquito infection levels following feeding of the vector on *P falciparum* gametocytes grown *in-vitro*. Screening candidate by SMFA is hampered by the requirement for human serum in the gametocyte culture medium and the variability in parasite growth observed between batches of non-immune human serum obtained from different donors. The replacement of serum with a more chemically defined material

would be beneficial, both for drug development and also for research work on transmission biology of *P falciparum*. In this study we investigated the effect of lipid supplementation of serum free medium on the development and infectivity of *in-vitro* cultured *P falciparum* gametocytes. We have tested a variety of commercially available lipid mixtures and individual lipids and fatty acids for toxicity to parasites and capacity to support growth. Successful supplements were then tested for their capacity to support gametocyte development and infectivity to mosquitoes. Our results are encouraging for the replacement of serum in *P falciparum* growth medium.

## 16 Anti-Mullerian hormone but not rapamycin attenuates both cyclophosphamide-induced damage and PI3K signalling activation in human ovarian cortex

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**Introduction** Cyclophosphamide is proposed to cause primordial follicle loss directly through follicle damage and indirectly by eliciting premature primordial follicle activation via PI3K signalling however most available data are from rodent models. Here we determine the effects of *in-vitro* cyclophosphamide exposure on the human ovarian cortex.

**Methods** Human ovarian cortical tissue from healthy women (aged 29-40yo) was cultured with 0.2μM (low dose) and 2μM (high dose) 4-hydroperoxycyclophosphamide (4-HC, the active form of cyclophosphamide). Rapamycin (100nM) and anti-Mullerian hormone (AMH) (200ng/μL) were explored as chemoprotectants. Histological analyses, Western blotting and immunohistochemistry were undertaken to assess follicle health and PI3K signalling activation (n=4-7). scRNA-seq was used to profile vehicle and 4-HC-exposed ovarian cortical transcriptomes (n=3).

**Results** Exposure to 4-HC increased the proportion of unhealthy primordial ($p<0.0001$, both doses) and transitional follicles ($p<0.003$ low dose, $p<0.005$ high dose). Culture with AMH significantly reduced follicle damage in both 4-HC doses investigated ($p<0.0001$); rapamycin had no effect. PI3K signalling was activated following 4-HC exposure as evidenced by increased AKT phosphorylation ($p<0.009$ low dose, $p<0.03$ high dose) and increased phosphorylated FOXO3A immunostaining ($p<0.0001$, both doses). AMH reduced both AKT phosphorylation and phosphorylated FOXO3A staining intensity across both doses investigated; rapamycin was only effective in low dose experiments. scRNA-seq data showed that 4-HC exposure affected the expression of cell damage, inflammatory response, apoptosis and cell cycle-related genes.

**Conclusion** Exposure to 4-HC compromises the health of primordial and transitional follicles in the human ovarian cortex and upregulates PI3K signalling. AMH, unlike rapamycin, consistently ameliorates both of these chemotherapy-induced effects.

## 17 IL-33 in intestinal mucosal immunity

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**Introduction** IL-33 and its receptor ST2 is known to impact fibrosis. In 30% of Crohn's disease patients, inflammation causes accumulation of scar tissue that eventually requires surgery. The effects of IL-33 on macrophages and the gastrointestinal epithelial barrier were assessed.

**Methods** Ethical approval was granted for collection of blood from volunteers and human terminal ileum (TI) tissue from Tayside biorepository.

CD14+ monocytes were isolated and differentiated into M0 macrophages using 10% autologous human serum over 7-10 days, then differentiated into M1 macrophages using LPS(100ng/ml) +IFN-γ(100ng/ml), or M2 macrophages using IL-4(20ng/ml) +IL-13(20ng/ml) for 24 hrs +/- IL-33 (100ng/ml) for a further 24hrs. Cells were phenotyped using flow cytometry(CD14, CD68, CD80, CD209, ST2), by IHC(CD68, ST2), gene expression (n=5) of 9 genes were assessed by RT-qPCR.

TI tissue was dissociated with 5mM EDTA and shaken to release the intestinal crypts. These were cultured in Matrigel and growth media over 6 weeks into mature organoids, then stimulated +/- TNF-α(100 ng/ml) + LPS(500 ng/ml) and +/- IL-33(100ng/ml). Gene expression (n=5) was assessed in a panel of 21 genes by RT-qPCR.

**Results** CD68+ macrophages express phenotypic markers (M1:CD80, IL-1β; M2:CD209, CD206). No differential gene expression was observed in response to IL-33. ST2 was not observed by flow cytometry, gene expression, or IHC.

TI organoids express ST2 and genes for epithelial barrier permeability, immunity, and fibrosis but show no differential expression in response to IL-33.

**Conclusions** Human macrophages do not express ST2. No differential gene expression in human TI organoids in response to IL-33 was observed.

## 18 Discovery of DYR726, a brain-penetrant selective inhibitor of PI3K, PDGFR, and the WNT pathway

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Glioblastoma (GBM) is a highly invasive brain neoplasia with a median patient survival of 12-15 months from initial diagnosis. The highly refractory and heterogenous nature of GBM is primarily attributed to the large population of glioma stem cells (GSC) which exhibit remarkable plasticity and drug-resistance. Hence, to date, all repurposed kinase inhibitors exhibiting decent blood-brain-barrier penetrance failed glioma clinical trials. Loss of key tumour suppressors like PTEN and NF1 coupled to oncogenic activation of receptor tyrosine kinase PDGFRA and lipid kinase PIK3CA drive proliferation and invasiveness in GBM. Furthermore, the WNT-β-catenin signalling pathway maintain glioma stem plasticity through transcriptional upregulation of MYC, SNAIL, SOX2, NANOG. Hence, a successful therapeutic strategy will require pleiotropic targeting of diverse signalling pathways in glioma which promote proliferation and plasticity. As such, over the last two years, our international team has embarked on a medicinal chemistry project, screened over 250 molecules, and established a blood-brain-barrier penetrant first-in-class PIK3CA/PDGFRA/WNT pathway inhibitor with a goal to start clinical development by 2026-27 for glioma therapeutics. DYR726 has been benchmarked against clinically relevant kinase inhibitors including avapritinib, abemacicilib, osimertinib, crenolanib and exhibits a superior *in-vitro* biological profile in targeting a panel of primary patient-derived adult and paediatric glioma cells and 3D GSCs. Importantly, DYR726 exhibits a therapeutic window of 10-fold between GSCs and normal neurons suggesting potential glioma specificity. Although kinase inhibitors have not been successful in targeting glioma in the clinic, the pleiotropic nature of our molecule could indeed be an interesting new development and a potential new chapter for glioma therapeutics.

## 19 Development of novel polymer-drug conjugates: Towards a multi-target treatment for Alzheimer's disease

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Development of novel polymer-drug compounds is urgently required to improve the ability to deliver Alzheimer drugs in the body. This project aims to design novel antioxidant polymer-drug conjugates (PDCs) to treat Alzheimer's disease (AD).

**Methods** An antioxidant drug (using various % attachments: 10%, 15%, 20%, and 100%) was conjugated to a constant amount of cationic polymer by a condensation reaction and characterised by NMR and FTIR. The antioxidant activity was analysed *in-vitro* using the ABTS assay. Cell viability and cellular protection from oxidative damage and inflammation by PDCs were determined in SHSY5Y and BV-2 cells *in-vitro* using an MTT assay.

**Results** Successful conjugation of novel PDCs (NM10, NM15, NM20, NM100) was shown with 1H NMR and FTIR. Using the ABTS antioxidant assay, NM10, NM15, and NM20, significantly increased antioxidant activity ($IC_{50}$= <20 µg/mL) compared to the control antioxidant ($IC_{50}$= >10 mg/mL) and polymer alone ($IC^{50}$= >40 6 µg/mL) ($p \leq 0.0001$). PDCs were toxic to both cell lines (<80% cell viability), so a polyelectrolyte complex (PEC) was developed. PEC NM15 significantly protected SH-SY5Y and BV-2 cells from oxidative damage. Results showed >30% and >40% protection against $H_2O_2$ in SH-SY5Y and BV-2 cells ($P \leq 0.0001$), respectively, as well as >35% protection against Rotenone/Antimycin A in SH-SY5Y ($P \leq 0.0001$) and >25% protection in BV2 cells ($P \leq 0.01$) against Lipopolysaccharide-(LPS) induced inflammation.

**Conclusion** This work has demonstrated the possible use of PDCs for the future development of a multi-target treatment option for AD.

## 20 Alterations in GPCR regulation after high fat diet feeding

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**Introduction** Obesity has reached epidemic proportions worldwide and accelerates Type 2 diabetes (T2D) and non-alcoholic fatty liver disease development, hence an urgent need for new therapeutics. We investigated how short term (ST) and long term (LT) high fat diet (HFD)-feeding impacts expression of the highly druggable G-protein-coupled receptor (GPCR) family in male and female mice.

**Methods** Animal procedures were performed under a project licence approved by the UK Home Office under the Animals (Scientific Procedures) Act 1986/ASPA Amendment Regulations 2012. We used 8-week-old male and female C57BL/6 mice (n=24/group) fed either chow or HFD for 4 or 16 weeks. Hepatic gene expression was assessed using GPCR-specific DNA microarrays and RT-qPCR and protein by Western blotting.

**Results** Males exhibited increased weight gain and blunted glucose homeostasis after ST HFD-feeding whereas these occurred later in females. In addition, hepatic steatosis was more pronounced in males. Genetic analysis revealed 210 GPCR-encoding genes altered by HFD-feeding, although largely consistent between sexes except for *Ptgfr* that was upregulated only in females ($p \leq 0.001$). Both sexes exhibited decreased β-arrestin 1/2 protein following ST HFD-feeding ($p \leq 0.01$) whereas GPCR-regulating kinase (GRK2) was elevated after LT HFD exposure compared to chow controls ($p \leq 0.05$ and $p \leq 0.01$).

**Conclusions** Our data reveal male mice become obese, develop T2D and hepatic steatosis faster than females. Importantly, genetic analysis revealed targets for exploitation and highlight both length of diet exposure and gender can impact gene expression. Finally, we reveal changes in β-arrestin 1/2 and GRK2 protein suggesting therapeutics targeting GPCR regulating machinery could be beneficial.

## 21 Pioglitazone modelling as a secondary prevention strategy after ischaemic stroke

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**Aims** Pioglitazone is recommended for secondary prevention after stroke in diabetics or insulin resistant people, yet its use remains low. We explored the potential impact of pioglitazone.

**Methods** We included people admitted with non-cardioembolic ischaemic stroke or TIA who survived to discharge in NHS Greater Glasgow & Clyde between 01/12/2015 and 31/12/2018. People were eligible for pioglitazone if they had type 2 diabetes mellitus or insulin resistance (mean HbA1c ≥42mmol/mol or obesity), no heart failure and not receiving pioglitazone.

We calculated event rates and applied a treatment effect based on the IRIS trial to those eligible (reduced by ½ to estimate real-world impact). Absolute risk reduction (ARR) and number needed to treat (NNT) to prevent stroke or stroke and myocardial infarction (MI) for those eligible were calculated.

**Results** The study included 4037 people: mean (SD) age 68.6 (12.9) years, 49% female. During median follow up of 2.2 years, 522 (12.9%) had recurrent stroke and 615 (15.2%) had stroke or MI. 1587 participants (39.3%) were eligible for pioglitazone, 162 (10.2%) had recurrent stroke and 192 (12.1%) had stroke or MI. We estimate there would be 147 (95% CI 130-170) recurrent strokes and 169 (95% CI 156-185) strokes or MI in this group with pioglitazone therapy. This gives an ARR for stroke or MI of 1.45% (95% CI 0.42-2.30%) with NNT 69 (95% CI 43-233) in treated people.

**Conclusions** Using real-world data, we estimate that pioglitazone use would prevent a small but important number of stroke and MI in a Scottish population.

## 22 The impact of a Western diet on the cardiovascular system of a transgenic mouse model expressing the single-nucleotide polymorphism (SNP), rs713041, in the human selenoprotein glutathione peroxidase 4 3' untranslated region

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The single-nucleotide polymorphism (SNP), rs713041, located at position 718 of the glutathione peroxidase 4 (GPX4) mRNA in the 3' untranslated region (3'UTR), has been shown to be functional in regulating GPX4 synthesis, and substitution of the major allele C into the minor allele T has been reported to be linked to stroke and hypertension. The combined impact of a Western diet (WD) and the SNP, rs713041, on the cardiovascular system in a human GPX4 3'UTR knock-in mouse model was thus investigated.

Male/female Tuko52 (C allele of rs713041) and Tuko51 (T allele of rs713041) mice were fed either a standard diet (SD) or a WD (high fat chow and 30% fructose in drinking water) for 12 weeks. In response to the WD, Tuko52 mice exhibited enhanced weight gain and adiposity compared to Tuko51 mice. Conversely, WD fed male Tuko51, but not Tuko52, mice had increased heart weight:tibial length, while a similar effect was observed in WD female Tuko52 mice. BP was not

altered in response to either genotype or dietary intervention, however endothelial-dependent vasorelaxation was impaired in arteries from male Tuko51 versus Tuko52 mice (SD), and in male Tuko52 in response to a WD. Finally, the expression of both fibrotic and inflammatory markers was increased in the hearts of male mice in response to the WD.

This study demonstrates that there are both organ- and sex-specific effects of the human GPX4 (rs713041) SNP in response to a WD, which are dependent on the presence of either the C or T allele.

## 23 Towards rapid detection of microplastic particles in human blood samples

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Small particles of plastic, called micro/nano plastics, have been found in all environments, including oceans, land, freshwaters and within human blood, lungs and liver. The long-term human health impact of these micro/nano plastics is still unknown, but several reports suggest that the presence of plastic inside humans leads to inflammation, oxidative stress and potentially organ damage. With plastic pollution growing relentlessly, understanding the fate and impact of micro/nano plastic pollution on humans is of paramount importance, but the techniques used to detect the presence of these plastics is difficult and time consuming.

In this project we aim to address these limitations through development of a point-of-use device to detect micro/nano plastics in blood without the need for expensive laboratory expertise or equipment. Peptide sequences have been synthesised with binding affinity to polystyrene (PS) particles. These sequences have then been tagged with an enzyme. In the presence of a colorimetric substrate for the enzyme, it is then possible to detect the presence of peptide bound to PS, and hence the plastic itself.

To complete the system for application as a point-of-use device, we have further proposed to demonstrate detection with lower cost electrochemical instrumentation, and to integrate with a miniaturised separation platform to remove unbound tagged peptide and other large particles, such as platelets and cells, from the sample.

## 24 Investigate retinal cholesterol homeostasis in an Alzheimer's disease mouse model

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Alzheimer's disease (AD) is the most common cause of senile dementia. Increased levels of Aβ peptide have been reported in the retinas of AD patients. Cholesterol has been reported to interact with Aβ42 possibly via the region of residues 22-35 in the Aβ sequence and modulating cholesterol level can regulate Aβ aggregation. Activation of CYP46A1, an enzyme controlling cholesterol elimination from the brain, reduces Aβ burden and improves long-term spatial memory in 5XFAD mice. The 5XFAD mouse model overexpresses human mutant amyloid precursor protein and mutant presenilin 1 and recapitulates major features of AD amyloid pathology. 5XFAD mice also show photoreceptor degeneration and RPE cell atrophy. Cholesterol also increases Aβ production in cultured retinal pigment epithelial (RPE) cells and lowers activity and expression of Aβ degradation enzymes in RPE cells. The 18 kDa translocator protein (TSPO) is localized to the outer mitochondrial membrane of different tissues. Its major function is transporting cholesterol from the mitochondrial outer to inner membrane where cholesterol is metabolized into pregnenolone by Cyp11A in steroid-producing cells or into oxysterols by CYP27A1 in non-steroidogenic cells. We have shown that TSPO mediates cholesterol transport in human RPE and choroidal endothelial cells and that loss of TSPO in human RPE cells results in abnormal accumulation of intracellular cholesterol, and demonstrated that TSPO ligands promoted cholesterol efflux in RPE and choroidal endothelial cells, upregulated expression of cholesterol homeostasis genes, and suppressed oxLDL-induced oxidative stress and inflammation. Investigation of TSPO ligand, Etifoxine on 5XFAD mouse model shown to reverse retinal pathology.

## 25 Lung stromal cell dynamics are altered by infection experience and ongoing antigen presentation following influenza challenge

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**Introduction** Stromal cells can be permanently altered by insults, a process termed trained immunity. Whether these cells contribute to protection or pathology in infections such as influenza a virus (IAV) is unclear. We hypothesise that trained stromal cells may participate in protective immunity by rapidly reactivating local memory T cells.

**Methods** We performed transcriptional analysis on sorted lung epithelial cells and fibroblasts isolated from naïve and IAV infected mice (primary, memory, and re-challenge timepoints). Stromal cell dynamics and interactions with immune cells were investigated using flow cytometry and immunofluorescence including detecting infected cells via IAV Nucleoprotein (NP). The location of infection experienced stromal and immune cells in the lung was determined using RNAscope.

**Results** RNA-sequencing analysis demonstrated enrichment in immune related genes (MHCII and CXCL9/10) at primary/memory timepoints in lung stromal cells. These genes were further upregulated following IAV re-challenge. Importantly, IAV-nucleoprotein+ epithelial cells expressed more MHCII compared to IAV-NPnegative cells, suggesting enhanced communication with T cells. Using RNAscope, SpiB, a transcription factor that regulates genes involved in antigen processing/ presentation, was detected in lung epithelial cells of infected mice. SpiB+ cells were in close proximity to immune cells that form dense clusters containing a mixture of T/B cells and myeloid populations. Interestingly, these microenvironmental changes were dependent on viral replication.

**Conclusions** Infection experienced stromal cell subsets may promote immune protection upon re-infection through antigen presentation to T cells and/or alteration of the local lung microenvironment. Increased understanding of stromal cell trained immunity may enhance our ability to protect against respiratory infections.

**NOTES**

## SHARE – Scottish Health Research Register and Biobank

SHARE is a Scottish research database of over 300,000 willing volunteers (aged 11+) who agree to allow the use of their health records to check their eligibility for research projects. In addition, most registrants also agree to the use of left-over blood following routine tests to be retained for anonymised genetic research. There are over 100,000 stored full blood or plasma samples.

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