RDM Day 2025

Monday 17 March

Mathematical Institute

A day to celebrate our science and build a culture in which all can thrive

Radcliffe Department of Medicine

UNIVERSITY OF OXFORD

Programme

8.45-9.15	**Registration, with tea and coffee available**		
9.20-9.30	**Welcome and Introduction** (Lecture Theatre 1) Professor Keith Channon, Head of RDM		
9.30-10.10	**Panel Session: Departmental Review** (Lecture Theatre 1) Chaired by Professor Keith Channon		
10.15-10.55	**Coffee/Tea Break and Poster Sesion One** (Mezzanines)		
11.00-12.00	**Science Session 1: Cardiovascular and Metabolic Medicine** (Lecture Theatre 1) Chaired by Professor Ellie Tzima Professor Leanne Hodson: 'Dietary fat: where does it go?' Lightning Talks: • Gareth Purvis: 'Epigenetic memory in hematopoietic stem cells following elevated cholesterol' • Daniel Rosoff: 'Human genetics and causal genes for cardiometabolic and clock/sleep disorders' • Kenneth Chan: 'Coronary inflammation and personalised cardiovascular risk prediction with cardiac CT' Graduate Prize Talk by Jamie Kitt: 'Cardiovascular risk following hypertensive pregnancy: the impact of postpartum blood control'		
12.00-13.25	**Lunch and Poster Session Two** (Mezzanines)		
13.30-14.15	**Parallel Sessions**		
	Why every lab needs a handbook **Maddie Mitchell will facilitate this session on research culture** **Lecture Theatre 3** *Chair: Associate Professor Naveed Akbar*	**Sharing research technologies across RDM** **Showcasing some of the facilities available for researchers to use** **Lecture Theatre 1** *Chair: Associate Professor Bethan Psaila*	**Managing your career** **Susan Black from the Careers Service provides hints & tips to help you take charge of your career** **Lecture Theatre 2** *Chair: Professor Thomas Milne*

14.20-15.20	**Science Session 2: Molecular Medicine** (Lecture Theatre 1) Chaired by Professor Graham Ogg Professor Simon Davis: 'A new way to study signalling pathways in T cells' Lightning Talks: • Agne Antanaviciute: 'Spatially resolved insights into fistulating Crohn's Disease pathogenesis' • David Cruz Hernandez: 'High thrombopoietin restricts preleukemic proliferation to the foetal liver in Down's syndrome' • Jakob Haldrup: 'Traceless delivery of large genome editing toolkits' Graduate Prize Talk by Charlotte Brierley: 'From genomic shattering to leukaemia: chromothripsis in blast phase MPN'
15.25-16.10	**Afternoon Tea and Poster Session Three** (Mezzanines)
16.10-16.15	**Concluding Remarks and Prizes** (Mezzanine - see map) Professor Keith Channon

NB Everything happening in Lecture Theatre 1 will be live streamed to Lecture Theatre 4 (see map on back cover). You may find that this is a less crowded room.

During the breaks, you may wish to talk to representatives from the following, who will be stationed at reserved tall tables:

- **The RDM Student Forum** relays feedback and concerns students may have about their course, and organises activities to bring the student body together across RDM.
- **The RDM Researcher Association** is run by RDM researchers, for RDM researchers and fosters community through social, networking, and career development events.
- **Oxford University Careers Service** supports students, alumni and research staff to navigate the complex job market and make informed career decisions.
- **Oxford University Innovation** provides academics, staff and students from across the University with advice and support on the commercialisation of research and expertise.
- **The RDM Communications Team** can help you promote your research, publicise your event, advertise your study, set up webpages, use social media and engage with others.

Poster number: 1

“Bug-to-Drug”: Tick-inspired treatments for Type 1 Diabetes

Authors: Megan Payne (DPhil Student)[1], Ali Shilleh[2], David Hodson[2] and Shoumo Bhattacharya[1]

Affiliations: 1 – Cardiovascular Medicine, 2 – OCDEM. Radcliffe Department of Medicine, University of Oxford.

Rationale: In type I diabetes, T-cells migrate to islets within the pancreas and destroy insulin-producing cells. Drugs suppressing T-cells delay disease onset but are associated with infection and cancer. Suppressing chemokine-mediated T-cell trafficking is an attractive strategy but is foiled by redundant chemokine signalling. Ticks produce “evasin” proteins that bind multiple chemokines, overcoming redundancy. Evasins are potentially immunogenic, making them unsuitable as therapeutics. Peptides overcome this limitation. We hypothesised that evasin-peptides that overcome chemokine redundancy could be identified from tick evasins.

Methodology: Phage-displayed evasin-peptides were selected by binding to multiple chemokines and identified by next-generation sequencing. This was followed by saturation mutagenesis to identify improved variants. AlphaFold3 was used to model peptide:chemokine interactions. Boyden chamber chemotaxis assays were performed to assess the potency of peptides in blocking $CD8^+$-T-cell migration to individual chemokines and to a 19-chemokine mix that models RNA-sequencing-derived expression in inflamed islets.

Results: We identified a multi-chemokine-binding peptide, EB429, that significantly inhibits migration of $CD8^+$-T-cells to chemokines CXCL9, CXCL10, CXCL11, CXCL12α, and CCL19. Saturation mutagenesis identified several substitutions with improved multi-chemokine binding and de novo chemokine inhibition. A tryptophan-substituted peptide significantly inhibited $CD8^+$-T-cell migration to an inflamed islet 19-chemokine pool. AlphaFold3 analysis showed that the tryptophan substitution enhances hydrophobic bond formation, providing a mechanism.

Conclusions: A combination of natural selection in “bugs” and lab-based evolution has allowed us to identify a drug-like peptide that can overcome islet chemokine redundancy. We suggest that targeting such peptides to native and transplanted islets will reduce T-cell trafficking and beta-cell death without adversely impacting the immune system. Future work will focus on engineering islets to produce peptides in situ.

Poster number: 2

Is activation and expansion of mediastinal lymph nodes a contributory disease mechanism in patients with hypertrophic cardiomyopathy?

Authors: Jacky C. K. Fung[1,2]* (DPhil), Kamayani Singh[1,2], Sunitha Balaraju[1,2], Alexander Sparrow[1], Violetta Steeples[1], Paul Robinson[1], Andrew Blease[1,2], Sarosh Irani[3], Alexander Clarke[4], Betty Raman[1], Houman Ashrafian[1,5], Charles S. Redwood[1], Ying-Jie Wang[1,2], Hugh Watkins[1,2]

Affiliations: [1]Department of Cardiovascular Medicine, Radcliffe Department of Medicine, [2]Centre for Human Genetics, [3]Nuffield Department of Clinical Neurosciences, [4] Kennedy Institute of Rheumatology, [5]Experimental Therapeutics, University of Oxford

Hypertrophic cardiomyopathy (HCM) is a familial disease often caused by single sarcomeric mutations and characterised by myocyte hypertrophy, disarray, and interstitial fibrosis. Although the majority of patients have a relatively benign course, HCM can lead to sudden cardiac death and heart failure. Life-long adverse remodelling occurs in many patients, some of whom may develop a severe stage, burned-out HCM. Although the mutation-induced contractile and energetic alterations are well documented, the mechanisms that lead to burned-out HCM remain poorly defined. To understand the pathogenesis of burned-out HCM, a well-characterised HCM mouse model (Actc1E99K/+) that recapitulates the clinical findings, including extensive fibrosis and reduced contractility, was employed. The heart-draining mediastinal lymph nodes (MedLNs), but not other lymph nodes, exhibited progressive expansion in parallel with adverse remodelling and lymphocyte accumulation in E99K hearts. Furthermore, lymphocyte activation and germinal centre formation were noticed in enlarged E99K MedLNs. Cardiac magnetic resonance (CMR) image analysis revealed a significant proportion of HCM patients (31%, 66 in 214) with increasing number and size of MedLNs in comparison to that of healthy controls (13%, 4 in 30), suggesting that a specific adaptive immune response may exist in some HCM patients. A preliminary in-vivo study was performed by adoptive transfer of late-stage E99K MedLN-derived cells (45+ weeks) into early-stage E99K mice (16 weeks) to examine their pathogenicity. Those donor cells significantly worsen contractile dysfunction in recipient mice, suggesting a potential role for adaptive humoral immunity in HCM. If confirmed, our results may identify a therapeutic target for reducing progression to burned-out HCM.

Poster number: 3

daLUXendins: novel dual GLP1R/GIPR fluorescent probes to advance diabetes and obesity research

Authors: Anne de Bray[1,2*], Jason Tong[2], Christiane Huhn[3], Kilian Roßmann[3], Ali H. Shilleh[2], Wanqing Jiang[4], Anna G. Roberts[4], Katrina Viloria[1,2], Daniela Nasteska[1,2], Abigail Pearce[5], Satsuki Miyazaki[6], Jeremy W. Tomlinson[2], Dylan M. Owen[7,8,9], Daniel J. Nieves[7,8,9], Julia Ast[1,11], Malgorzata Cyranka[11], Alexey Epanchintsev[11], Carina Ämmälä[11], Frank Reimann[10], Graham Ladds[5], Alice Adriaenssens[4], Stefan Trapp[4], Ben Jones[12], Johannes Broichhagen[3]*, David J. Hodson[1,2]

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*Presenting author, DPhil student

Rationale: Tirzepatide is a novel medication for type 2 diabetes and obesity, showing dramatic weight loss and improved glycaemic control in patients. It is a biased dual glucagon-like peptide 1 receptor (GLP1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR) agonist. GIPR/GLP1R activation is key in post-prandial glucose control but the cell targets for tirzepatide's biased agonism remains unclear.

Methodology: Cell-specificity and cell/tissue targeting of two novel fluorescent tirzepatide-based probes (daLUXendin544/642) were assessed with confocal/dSTORM microscopy in a range of relevant live and fixed tissue settings.

Results: Both probes demonstrated GIPR/GLP1R co-localisation with cells expressing tagged receptors or stained for insulin, glucagon and somatostatin. The probes demonstrated reduced fluorescence in GLP1RKO islets versus WT islets, with further reduction when co-treated with excess GIPR agonist. Systemic administration of daLUXendin642 demonstrated co-localisation with Glp1r-Cre:tdRFP and Gipr-Cre:GFP positive pancreas cells and strong labelling in circumventricular organs characterized by an incomplete blood-brain barrier. Upon intracerebroventricular administration, daLUXendin642 co-localises widely with GLP1R+ and GIPR+ neurones, with evidence of uptake by 3rd ventricle-lining cells presumed to be tanycytes. Single molecule imaging of daLUXendin642 demonstrates targeting of endogenous GLP1R-GIPR nanodomains, which differ in organisation and composition to those targeted by single agonist.

Conclusion: Diabetes and obesity are chronic diseases with significant morbidity and mortality and dual incretin agonists represent the next generation of treatments. daLUXendins are specific for GLP1R and GIPR in several relevant tissue settings and reveal dual agonist targets in the pancreas and brain.

Poster number: 4

VZV-ORF9 Acts as a Histone Mimic to Inhibit cGAS

Authors: Gerissa Fowler[1](DPhil), Jonny Hertzog[1], Jan Rehwinkel[1]

Affiliations: [1]MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Rationale: Varicella-Zoster virus (VZV) causes chickenpox and shingles. The molecular mechanisms that determine innate immune responses remain poorly defined. Previously, we found that the cGAS protein, a receptor of the DNA sensing pathway, is inhibited by the VZV-ORF9 protein (Hertzog et al., EMBO J 2022). However, the mechanism of cGAS inhibition remained unknown. cGAS is bound and inhibited in the nucleus of cells by binding to the acidic patch of the nucleosomal protein dimer H2A-H2B. An amino acid sequence alignment of VZV ORF9 and H2A revealed sequence similarity to H2A's acidic patch region.

Methodology: HEK293T, THP1, and HFF cells were transiently transfected or transduced to express ORF9 and ORF9 mutants. The introduced mutations revert the negative charge of amino acids in ORF9's putative acidic patch to no longer retain similarity to histones. ORF9 overexpressing cells were stimulated with dsDNA and the type I IFN response was measured via RT-qPCR and ELISA.

Results: ORF9 mutant overexpressing cells stimulated with DNA induced an ISG response similar to that of GFP overexpressing control cells. Compared to ORF9 WT, which supresses ISG induction via cGAS inhibition, the ORF9 mutants lost the ability to supress ISG/IFN induction, potentially via loss of cGAS binding. The loss of function in ORF9 mutants indicates that this viral protein may share the cGAS inhibition mechanism with histones. Consistently, AlphaFold modelling and RMSD values indicate similarities of the secondary structure of ORF9 and H2A.

Conclusions: We propose histone mimicry as a strategy of cGAS inhibition by VZV.

Poster number: 5

Comprehensive characterization of tumor-specific CD8 T cells in HR+ breast cancer patients reveal an impaired antitumoral response in patients with lymph node metastasis

Authors: Mariana Pereira Pinho[1,2], Elie Antoun[2], Balraj Sandhar[1,2], Ting Shu[2], Fei Gao[1,2], Xiaobao

Yang[2], Lucia Cerundolo[3], David Maldonado-Perez[3], Renuka Teague[3], Adam Bates[1,2], Megat H. B. A.

Hamid[2], Eve Warner[3], Lucinda Winter[4], Nasullah Khalid Alham[5,6], Clare Verrill[3,6], Simon Lord[7], Timothy Rostron[1], Sally-Ann Clark[1], Craig Waugh[1], Paul Sopp[1], Chris Conlon[2], Ricardo A. Fernandes[2], Adrian L. Harris[7], Yanchun Peng[1,2], Asha Adwani[8], Tao Dong[1,2]

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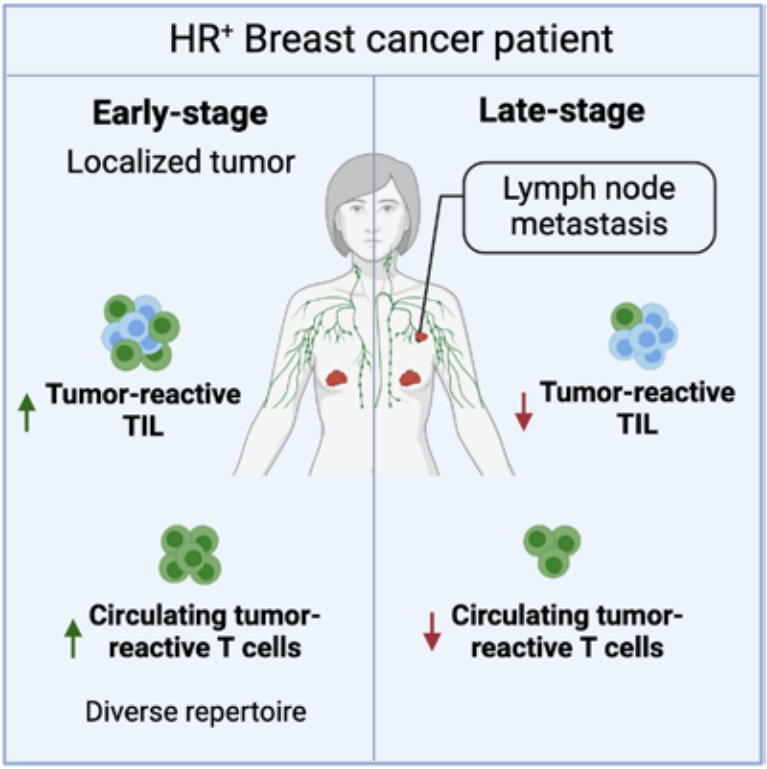
Most breast cancers express the estrogen receptor (ER), but the antitumor immune response of hormone receptor-positive (HR^+) breast cancer remains poorly characterized. Here, dendritic cells loaded with tumor lysate were used to identify tumor-reactive CD8 T cells, which were detected in most HR^+ breast cancer patients, especially those with early-stage tumors. When present, the circulating antitumor CD8 response contained cytotoxic T cells with diverse specificity and TCR repertoire. Additionally, patients with blood cancer-specific T cells had significantly more CD8 tumor-infiltrating lymphocytes (TILs). Moreover, tumor-specific TCR sequences were found in the tumor, but at a significantly lower proportion in patients with lymph node involvement. Our data suggest that HR^+ breast cancer patients with lymph node metastasis lack tumor-specific CD8 T cellswith capacity to infiltrate the tumor at significant levels. However, early-stage patients have a diverse antitumor CD8 response that could be harnessed to develop immunotherapeutic approaches for late-stage HR^+ patients.


HR+ Breast cancer patient
Early-stage
Localized tumor
Late-stage
Lymph node metastasis
Tumor-reactive TIL
Tumor-reactive TIL
Circulating tumor-reactive T cells
Circulating tumor-reactive T cells
Diverse repertoire

Poster number: 6

Exploring the molecular mechanisms of altered calcitonin receptor signalling in atrial fibroblasts in patients with atrial fibrillation

Authors: Aaron Michael Johnston (DPhil Student)[1], Chi Him Kendrick Yiu[1], Lucia Moreira[1], Paul Robinson[1], Ileana Badi[1], Vivek Srivastava[2], Rana Sayeed[2], George Krasopoulos[2], Nicholas Walcot[2], Priya Sastry[2], Antonios Kourliouros[2], Nicola Smart[3], Svetlana Reilly[1]

Affiliations: (1) Radcliffe Department of Medicine (2) Department of Cardiothoracic Surgery, Oxford University Hospitals NHS Foundation Trust (3) Institute of Developmental and Regenerative Medicine, University of Oxford, United Kingdom

Introduction: Cardiac fibrosis is a major pathogenic mechanism in atrial fibrillation (AF). We recently uncovered that the heart produces the hormone calcitonin (CT), which, via binding to CT-receptors (CTR) in human atrial fibroblasts (hAFbs), regulates atrial fibrogenesis.[1] However, in AF, loss of physiological surface CTR precludes CT's beneficial effects. This study explored the molecular mechanisms underpinning this phenomenon.

Methods & Results: hAFBs were isolated from atrial appendage biopsies from patients in persistent AF or controls in sinus rhythm (SR). Immunostaining revealed enhanced CTR overlap with DAPI/reduced overlap with FLNa in AF-hAFbs (A, B). Meanwhile, cell fractionation and immunoblot confirmed an increased enrichment of CTR within the cytoskeletal/nuclear membrane subcellular compartments (C). The actin cross-linking protein FLNa has been reported to regulate CTR trafficking.[2] Co-immunoprecipitation confirmed a physical interaction between CTR and $FLNa^{CT}$ (D). Although full-length FLNa protein was non-significantly reduced in AF (immunoblot), there was a striking 53% reduction in the expression of FLNa C-terminal fragment ($FLNa^{CT}$), which contains the CTR-binding domain, in AF-hAFBs (E). Next, we explored whether changes in calpain-1 function/expression, known to cleave FLNa to generate $FLNa^{CT}$, can account for the reduced $FLNa^{CT}$ abundance. Although calpain-1 protein (F) was significantly (~75%) downregulated in AF, its activity, assessed by a fluorescence-based assay, remained unaltered (G). Furthermore, siRNA-mediated knockdown of calpain-1 had no effect on $FLNa^{CT}$ abundance (H). To assess whether enhanced proteolytic degradation contributes to reduced $FLNa^{CT}$ levels, SR-hAFbs were treated with proteasome inhibitor MG-132 which did not alter $FLNa^{CT}$ abundance (I). To explore alternative mechanisms, we performed mass spec analysis on CTR-immunoprecipitates from SR- and AF-hAFbs to identify protein interactors. GO cell component analysis revealed an enrichment of AF-specific CTR interactors within the nuclear lumen/nucleoplasm (J).

Conclusions: Persistent-AF is associated with aberrant CTR localisation and changes in the CTR interactome in hAFbs, accompanied by altered FLNa

processing, not attributable to changed calpain-1-mediated FLNa cleavage nor proteasomal degradation. Further characterisation of the molecular determinants of dysfunctional CTR localisation may uncover novel, potentially druggable, targets to minimise structural remodelling in AF.

Poster number: 7

Non-canonical Wnt signalling promotes vascular smooth muscle cell calcification via Complement 3

Authors: Ileana Badi[1*], Ioannis Akoumianakis[1], Jagat S. Chauhan[1], Sibel Kurt[1], Matthew Williams[1], Alejandro Cardona-Monzonís[1], Georgina Miles[1], Marianne Riering[1], Daniel Foran[1], Murray Polkinghorne[1], Christos P. Kotanidis[1], Rana Sayeed[2], George Krasopoulos[2], Vivek Srivastava[2], Nicholas Walcot[2], Antonios Kourliouros[2], Priya Sastry[2], Keith M. Channon[1], Charalambos Antoniades[1]

*postdoc

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Rationale: We aimed to study the poorly understood role of Wnt5a in vascular smooth muscle cell (VSMC) calcification.

Methodology: The study included 820 patients undergoing cardiac surgery. VSMC were isolated from vascular tissue biopsies while internal mammary arteries (IMA) were used for ex vivo experiments as well as RNA sequencing. Peri-IMA adipose tissue (PVAT), and pericardial adipose tissue (pericAT) were collected for gene expression studies; genotyping was performed using the UK Biobank Axiom Array and 10-year follow-up was completed.

Results: Inhibition of effectors (JNK-i and CaMKII-i) of the two non-canonical Wnt pathways prevented Wnt5a-mediated increase in calcium deposition and expression of VC promoters (i.e. RUNX2, BMP2, MSX2) in VSMC (A,B). Wnt5a increased JNK and CaMKII activation in IMA. WNT5A levels in IMA correlated with RUNX2. A bioinformatic analysis identified complement 3 (C3) as the most upregulated gene in Wnt5a-treated VSMC that also correlated in IMA with both WNT5A and RUNX2. C3 expression correlated with WNT5A also in IMA PVAT and pericAT. IMA levels of C3AR1, that is the gene coding for the C3a receptor, positively correlated with VC promoters' expression. C3 inhibition with either compstatin or a C3a receptor antagonist (C3-i) counteracted Wnt5a-induced VSMC calcium deposition (C). The single nucleotide polymorphism rs3745567 identified in a GWAS for C3 blood levels was associated with risk of cardiovascular events (D) and increased MSX2 IMA expression (E), that also associated with risk of cardiovascular events (F).

Conclusions: Non-canonical Wnt signalling promotes VSMC calcification through C3. This new pathway may be a therapeutic target in vascular dysfunction to reduce cardiovascular risk.

Poster number: 8

GC-globulin is a potent endogenous regulator of GLP1R signalling in pancreatic beta cells

Authors: Katrina Viloria[1] and Ali H. Shilleh[1], Ramona Birke[4], Greg Austin[2], Kaja Rupar[1], Jason Tong[1], Ildem Akerman[3], Johannes Broichhagen[4], Alejandra Tomas[2], Ben Jones[2], Martin Hewison[3], David J. Hodson Lab[1]

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Introduction: GC-globulin, a circulating fetoprotein and actin scavenger restricted to the liver and pancreatic alpha cells, regulates glucagon and insulin secretion. Given the dynamic role of the cytoskeleton in G protein-coupled receptor signaling, we hypothesized that GC-globulin might influence glucagon-like peptide-1 receptor (GLP1R) trafficking and signaling within the islet.

Methods: In mouse islets, F-actin and GLP1R trafficking were visualised using fluorescent microscopy. GLP1R signaling was determined using live cAMP imaging and insulin secretion assays. GLP1R-dependency was assessed using $GLP1R^{+/+}$ and $GLP1R^{-/-}$ islets. Anti-Flag co-immunoprecipitation was performed to identify physical interactions between GC-globulin and FLAG-tagged GLP-1R. Glucose tolerance of mice on standard chow and high fat diet injected with GC-globulin was assessed. RNA from islets treated with GC-globulin +/-GLP1R agonist (GLP1RA) was sequenced to determine changes in the beta cell transcriptome. Beta cell survival/apoptosis was determined using TUNEL assay.

Results: GC-globulin increased GLP1R surface expression, and prevented GLP1RA from internalizing GLP1R. Co-application of GC-globulin and GLP1RA amplified glucose-dependent cAMP-signaling and insulin secretion. Synergy between GC-globulin and GLP1RA was lost in islets deleted for GLP1R. GC-globulin co-purified with GLP-1R in both basal and agonist-stimulated conditions, and key ectodomain contact sites were predicted using AlphaFold 3 models. In mice, GC-globulin dose-dependently improved glucose tolerance. Anti-apoptosis, anti-ER stress and GPCR signalling were gene targets of GC-globulin supplementation. GC-globulin decreased beta cell apoptosis induced by glucolipotoxicity.

Conclusion: GC-globulin potently amplifies GLP1RA-stimulated cAMP signaling and insulin secretion through direct binding with GLP1R. The effects of GC-globulin improve GLP1R action, overall enhancing beta cell function and survival.

Poster number: 9

Iron deprivation counteracts systemic autoimmune inflammation caused by Treg depletion

Authors: Dana Costigan (DPhil Student), Hannah Murray, Giulia Pironaci, Alexandra Preston, Shamsideen Yusuf, Megan Teh, Andrew Armitage, Hal Drakesmith

Affiliation: Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, England

Iron is an essential micronutrient critical for innate and adaptive immunity as demonstrated by impaired responses to infection, inflammation and vaccination in iron deprived settings (Frost et al, 2022, Frost et al, 2021; Stoffel et al, 2020). We hypothesised that pharmacologic iron restriction could also impair maladaptive immune responses; in such a context this effect could inhibit autoimmune pathogenesis and potentially be of therapeutic benefit.

We used the FoxP3-DTR-GFP mouse model to conditionally deplete Tregs and induce systemic autoimmunity. Alongside Treg depletion-induced inflammation, we suppressed plasma iron concentrations with injections of minihepcidin, a peptide mimetic of the iron regulatory hormone hepcidin. We used high-dimensional flow cytometry and gene expression analysis to investigate impacts of iron restriction on inflammatory immune responses in multiple different organs.

We found that iron restriction has a profound systemic immunological effect, dampening splenomegaly and lymphadenopathy, restraining hepatic mRNA expression of serum amyloid A1 (Saa1), an acute phase protein and preferentially reducing T cell but not myeloid cell activation and proliferation. T helper cell activation, proliferation and subset frequencies were analysed in lung, liver, blood, spleen and lymph nodes showing global increases in T cell activation and increased Tbet+ and GATA3+ Thelper cell populations upon Treg depletion. Notably, T cell activation and Thelper cell populations were significantly reduced in majority of tissues upon mHep treatment, most profoundly in the lymph nodes. However in the spleen – the major site of iron recycling from senescent erythrocytes – minihepcidin had no effect on T cell activation, proliferation or T helper subset frequencies after Treg depletion, demonstrating tissue-specific effects of iron restriction treatment.

Our work shows that iron deprivation in the plasma compartment has the power to exert some of the T cell suppression otherwise performed by Treg cells and has therapeutic potential for dampening T cell mediated autoimmune disease.

Poster number: 10

Traceless delivery of large genome editing toolkits

Authors: Jakob H. Haldrup, Deborah Gill, Steve Hyde

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Genome editing holds significant promise for advancing genetic medicine, but current delivery methods face several challenges. By incorporating ribonucleoprotein (RNP) complexes into lentivirus-derived nanoparticles (LVNPs) instead of traditional vector genomes, we address critical limitations of both viral and non-viral delivery systems. These include: (i) overcoming limited packaging capacity, (ii) preventing unwanted genomic integration, and (iii) minimizing exposure to the therapeutic payload to minimize genotoxicity. The practical effectiveness of this LVNP platform has been demonstrated, enabling robust genome editing in both primary human T cells ex vivo and the murine retina in vivo (PMID: 37678882). We also showcased LVNP-mediated delivery of larger base/prime editors, although substituting Cas9 with larger RNP complexes led to reduced potency.

For clinical applications, ideal vectors must strike a balance between potency, safety, and manufacturability. To enhance LVNP potency, we added effector domains to support membrane anchoring during production and removed redundant viral proteins to improve packaging capacity. Additionally, we incorporated stem-loop aptamers to boost (e)pegRNA loading, which is the main limiting factor for prime editing efficacy. These modifications collectively resulted in a substantial improvement in prime editing efficacy, with a range of ~12-fold to ~48% across targeted loci. For manufacturability, we have established scalable, cGMP-compliant manufacturing protocols using VSV-G or Sendai F/HN pseudotypes. With these advancements, the LVNP platform is well-positioned for translational applications, aligning potency, safety, and manufacturability in preclinical studies involving patient-derived cells and in vivo models.

Poster number: 11

Integrative analysis of transcriptomics, proteomics and metabolomics to study mechanisms that regulate lentiviral vector hepatocyte transduction

Authors: Galina Boskh[1] (DPhil student), Kshitiz Tyagi [2], Andre Raposo [2], Stephen Hyde[1] , Shijie Cai[1] ,

Affiliations:

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Introduction: The liver is a key target for gene therapy due to its central role in metabolism and protein secretion. However, efficiently transducing quiescent adult hepatocytes with lentiviral vectors (LVs) is challenging, possibly due to intracellular factors limiting LV entry, integration, and transgene expression. We aim to investigate metabolic pathways to optimise the cellular environment and to enhance LV transduction in hepatocytes.

Materials and Methods: The transduction efficiencies of VSV-G pseudotyped, EGFP-expressing HIV- and SIV-derived LVs were compared in THLE-2 normal human hepatocytes, HepG2 hepatocellular carcinoma cells, and HEK293T cells. Proliferation rates, LV entry receptor density (LDLR), and genome integration (VCN), were assessed using Incucyte, Western blot, qPCR and ddPCR. Proteins, RNA, and metabolites from LV-transduced and non-transduced samples are to be prepared for multi-omics data analysis.

Results: HIV-LV achieved higher transduction than SIV-LV across all cell lines, with ~2-fold higher EGFP expression levels. However, transduction in THLE-2 cells was ~3-fold lower than in HepG2 and HEK293T, regardless of the LV used. Transduction did not affect cell viability or proliferation. In HepG2 cells, HIV-LV transduction resulted in a 2-fold increase in integrated VCN compared to SIV-LV. LDLR knockdown assays and epigenetic studies are ongoing to assess their impact on transduction. Furthermore, proteomics analyses of FACS-sorted LV-transduced and non-transduced cells are underway, with plans to extend to transcriptomics and metabolomics.

Conclusion: HIV-LV shows superior transduction efficiency compared to SIV-LV. Reduced transduction in THLE-2 cells suggests the presence of rate-limiting factors influencing LV entry, integration, or transgene expression, the mechanisms of which are being investigated using multi-omics analysis.

Poster number: 12

Extracellular Vesicle Dysregulation in Hypertrophic Cardiomyopathy

Waleed Seddiq1, Yiangos Psaras1, Amaury Genovese1, Leo Hesse1, Christopher Toepfer1, Naveed Akbar1

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Background: Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disorder primarily caused by mutations in sarcomeric proteins. Among these, the R145W variant in TNNI3 disrupts sarcomeric function, leading to myocardial remodelling and contractile dysfunction. Extracellular vesicles (EVs) are critical mediators of intercellular signalling and may play a role in HCM pathogenesis. This study employed an induced pluripotent stem cell (iPSC)-derived model of left ventricle-like cardiomyocytes (LV-like CMs) to examine structural and EV-related abnormalities associated with the R145W mutation.

Methods and Results: LV-like CMs were differentiated from wild-type (WT) and mutant (R145W) iPSCs using an optimised protocol. Genetic sequencing, immunofluorescence, and morphometric analyses confirmed CM identity and revealed significant hypertrophic remodelling in R145W compared to WT (1.5-fold increase in cell size, $P = 0.036$).

EVs were isolated using Size Exclusion Chromatography (SEC) from conditioned media collected between days 20-44 of differentiation. Characterisation using Nanoparticle Tracking Analysis (NTA), protein concentration, Transmission Electron Microscopy (TEM), and ExoView profiling of EV-markers CD9, CD63 and CD81 confirmed EV enrichment in SEC fractions 5-8, with fractions 1-4 and 9-11 containing extracellular particles. EV secretion was reduced in CM-R145W compared to CM-WT.

Conclusions: This study establishes a robust iPSC-based model to investigate EV dynamics in HCM. The observed EV secretion defects and altered biogenesis in R145W highlight potential disease biomarkers and mechanisms of intercellular communication. Future work will incorporate patient-derived cardiomyocytes and plasma EVs to validate translational biomarkers and elucidate the role of EVs in myocardial remodelling and HCM progression.

Funding: We acknowledge support from research grants provided by the British Heart Foundation (BHF) Centre of Research Excellence, Oxford (RE/13/1/30181 and RE/18/3/34214), the British Heart Foundation Project Grant (PG/18/53/33895), a British Heart Foundation Intermediate Fellowship (FS/IBSRF/22/25110) and the Royal Embassy of Saudi Arabia Cultural Bureau (SACB) research grant.

Poster number: 13

Evaluating the performance of the state-of-the-art class II ligand predictors in a biologically relevant context

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Class II ligands are peptides that bind to Human Leukocyte Antigen class 2 (HLA-II) molecules found on immune cells. This binding allows the immune system to recognize pathogens and the body's own proteins, triggering immune responses. HLA-II ligands are important because they interact with CD4 T-cells, a key immune cell type that modulates immune responses. Predicting HLA-II ligands allows us to identify which peptides are most likely to be presented by immune cells, guiding the development of targeted immunotherapies and improving our ability to modulate immune responses. Additionally, accurate predictions save time and resources by narrowing down potential candidates for laboratory testing, making research more efficient.

Recently, several HLA-II ligand prediction methods were published, claiming very high prediction performance [1-3]. However, our ongoing work and preliminary analysis indicate otherwise. This is in particular the case in a biologically relevant context where accuracy matters the most. In particular, we show that current methods do not account for post-translationally modified residues, despite their immunological significance and enrichment in disease-associated contexts. Furthermore, we identify problems with the methods' training and evaluation data, and the methods' overreliance on binding motifs. We also highlight that there is a weak method agreement for presumably high accuracy methods.

We support our arguments with statistical analysis of a large dataset of unseen peptides with known binding preferences to HLA-II molecules, including PTM peptides. Analysing the peptides, we show that PTM may alter a peptide's binding preference, while with error analysis of the methods, we identify avenues for their improvement.

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Poster number: 14

Using T-cell activation assays to study the diverse signalling of TIM-3

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Inhibitory receptors expressed on CD8 T-cells, such as PD-1 and CTLA-4, are thought to be responsible for the dampening of T-cell responses to chronic diseases, including cancer. Treatments which block ligand-receptor interactions of PD-1 and CTLA-4 have been partially successful in reversing this T-cell dysfunction. However, the signalling mechanisms of almost all inhibitory receptors are poorly understood. TIM-3 (HAVCR2) is highly expressed on 'terminally exhausted' CD8 T-cells in cancer and is thought to act as an inhibitory receptor on CD8 T-cells. The functions of TIM-3 have proven difficult to reveal and multiple signalling mechanisms have been proposed. Better knowledge of these mechanisms will enable new immunotherapies to reinvigorate dysfunctional T-cell responses.

We used a 2-cell TCR activation assay to study signalling in Jurkat reporter cells. Stimulation of these cells by A375 cells expressing a surface-bound OKT3 antibody allows measurement of T cell activation through an NFkB1-driven GFP reporter. By expressing a chimeric PD1ECD-TIM3ICD receptor on reporter cells, we studied TIM3 signalling in our reporter cells in both a tonic signalling and ligand-bound signalling context, by expression of PD-L1 on A375 stimulator cells. We have expanded this assay into reporter cells expressing a 1g4 TCR stimulated by A375 cells presenting NY-ESO peptide, to better replicate the immune synapse formed between T-cells and their targets. Our assays have revealed that TIM-3 has both activatory and inhibitory signalling potential depending on the cellular context, particularly CD3 levels on the cell. Future work will focus on altering TIM-3 signalling through use of chimeric TIM3 receptors and CRISPR knockouts to assess how changes in TIM-3 signalling affect T-cell activation.

Poster number: 15

Defining regulatory mechanisms for human cytotoxic CD4 T cells differentiation

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Although CD4 T cells helper functions are well described for anti- cancer immunity, there is increasing evidence for the direct role of cytotoxic CD4 T lymphocytes (CD4 CTLs) in tumour elimination. However, CD4 CTL differentiation, functional specialization, and therapeutic potential remain incompletely understood. We recently reported on the frequent existence of fully active, tumor-specific cytolytic CD4 T cells in cancer patientsa. Through ex vivo single-cell transcriptomic and spatial analyses of tumor-reactive CD4 T cells from paired blood and tumor samples of melanoma patients, we identified Killer Cell Lectin Like Receptor G1 (KLRG1) as a distinct surface marker of CD4 CTLs. KLRG1$^+$ CD4 CTLs required similar molecular pathways to classic CD8+ CTL, but killing occurred with distinct kinetics and frequency of contacts with target cells. KLRG1$^+$ CD4 CTLs exhibited enhanced tumor-killing ability compared to their KLRG1$^+$ counterparts; however, paired analyses indicated a depletion of this population at the tumour site versus circulation. Building on these findings, I am now seeking to define the phenotypic heterogeneity and differentiation mechanisms of CD4 CTLs in healthy donors. Using Infinity Flow unbiased high-dimensional cytometry analysis of >300 surface markers and genome-wide CRISPR activation and inhibition screens, I aim to identify novel markers of cytotoxic cellular states and key differentiation regulators. These insights will enable precise isolation and functional enhancement of CD4 CTLs, paving the way for their use in cancer immunotherapy.

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Poster number: 16

Dissecting the Phenotypic and Functional Heterogeneity of Human Naïve T Cells

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Peripheral T cell populations consist of a diverse assortment of heterogeneous cells which linked to cell fates with various functions, for example rapidly proliferating effector cells or long-lived memory cells [1]. Like the bone marrow, T cell populations have a structural hierarchy: naïve T cells at the apex, progressing through memory cells to terminally differentiated effector cells [2]. Early memory T cells used in immunotherapies such as chimeric antigen receptor T (CAR-T) cells, have demonstrated significant clinical benefits, including improved anti-tumour efficacy and persistence. However, relapse often occurs in treated leukaemia patients, and there are still unmet medical needs in treating solid tumours [3]. Naïve T cells, with their self-renewal abilities and multipotency, have great therapeutic potential. Crucial questions therefore are whether all naïve T cells the same, and if some subsets possess superior functional propensity that could overcome current treatment impediment? Recent evidence has shown that naïve T cells also exhibit heterogeneity [4]. However, comprehensive analysis of this diversity and its role in adaptive immunity remains unclear. The main goals of this project are to delineate the heterogeneity of naïve T cells through proteomic, transcriptomic, epigenetic, and functional characterisation, and to identify naïve T cell subpopulations with greatest therapeutic potential. Through machine-learning method, Infinity Flow [5], we unbiasedly explored the phenotypic heterogeneity of naïve T cells across different age groups (cord, young and old). By screening >300 surface markers, we identified a high level of naïve T cell heterogeneity and several new markers that segregated individual populations. 20+ markers selected from Infinity Flow were validated via spectral flow cytometry. Functional interrogation, including proliferation, differentiation profile, and cytokine secretion of 6 marker-defined subsets are currently underway. Understanding the diversity of naïve T cell may uncover their roles in adaptive immunity. Additionally, pinpointing subsets of naïve T cells with favourable therapeutic characteristics can improve manufacturing of cellular therapies for cancer treatment.

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Poster number: 17

Investigating the role of interleukin-11 (IL-11) in atrial fibrillation (AF)

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Background: Atrial fibrillation (AF), the commonest clinical arrhythmia, develops via atrial structural remodelling, hallmarked by fibrosis. Interleukin-11 has been implicated in fibrogenesis in the heart, lung and liver.1 However, its role in atrial fibrosis in the context of AF is less well known.

Methods: Human atrial tissue (left atrial) biopsies from 16 cardiac surgery patients in sinus rhythm (SR) or with persistent AF (AF) were used for human fibroblast isolation (standard enzymatic digestion protocol). Cells were cultured to passage 3-4. Gene and protein expression of IL11 and its receptor (IL11RA) were assessed by qPCR and western blot. Circulating and myocardium-secreted IL11 levels were evaluated in human serum samples collected from 18 patients undergoing electrophysiology catheter ablation; separated between AF and non-AF patient cohorts. Coronary sinus (CS) blood samples were collected using coronary sinus catheters. Secreted serum IL11 levels were measured with ELISA pre- and post-ablation, and at 3- and 12-month follow-up visits.

Results: Expression profiling studies (RT-qPCR) found that in human atrial fibroblasts (hAFB), mRNA expression of IL11 is significantly elevated persistent AF patients, while it was negligible in SR patients (Fig 1A). Immunoblotting revealed protein expression of IL11 receptor (IL11RA) in hAFBs was significantly upregulated (Fig 1 B,C). Similarly, myocardium-secreted levels of IL11 (from CS) displayed a trend towards elevation in patients with AF than non-AF controls prior to ablation (Fig 1D). This failed to normalise ~1.5 hours post-ablation, and were significantly elevated in those with AF compared to controls post ablation (Fig 1E). Furthermore, a striking correlation between circulating (measured peripheral venous) and myocardium-secreted (coronary sinus) IL11 serum levels (Fig 1F) is found.

Conclusions: Our results indicate that AF patients may display enhanced activation of IL11 signalling in the atrial myocardium, associated with elevated atrial IL11 gene expression and its receptor (IL11RA) protein abundance. We also demonstrate that IL11 is secreted by atrial myocardium, levels which positively correlate with circulating (peripheral vein (PV) derived) IL11

concentration, indicating that IL11 measured in PV blood samples can serve a good marker of the myocardial (CS) IL11 levels. Future studies will investigate impact of IL11 signalling activation/inhibition in hAFBs in context of AF.

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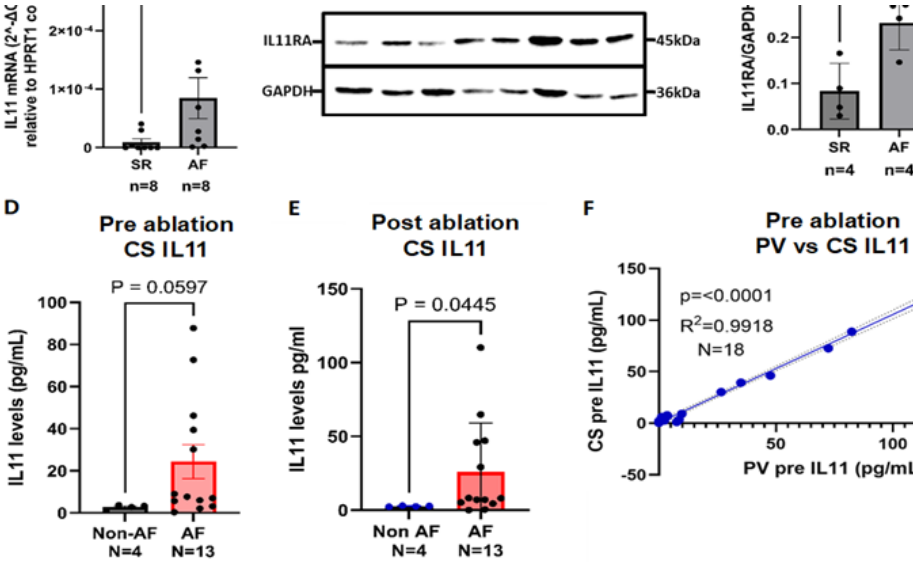

Figure 1.A) *IL11mRNA (*RT-qPCR) expression in atrial fibrillation (AF) versus sinus rhythm (SR) cont (normalized to *HPRT1)* in AFBs *(Stats: Mann-Whitney U test)*. **B)** IL11 receptor (IL11RA) in human AFBs (western blot) between SR and AF groups, quantified in C); *(Stats: unpaired t-test)*. **D)** Coron sinus (CS) IL11 levels measured by ELISA in serum samples prior catheter ablation, and 1.5hr post ablation in **E)**; (Stats: *Mann-Whitney U test)*. **F)** Correlation between peripheral venous (PV) and myocardial-secreted (CS) IL11 levels, measured by ELISA *(Stats: paired t-test)*.

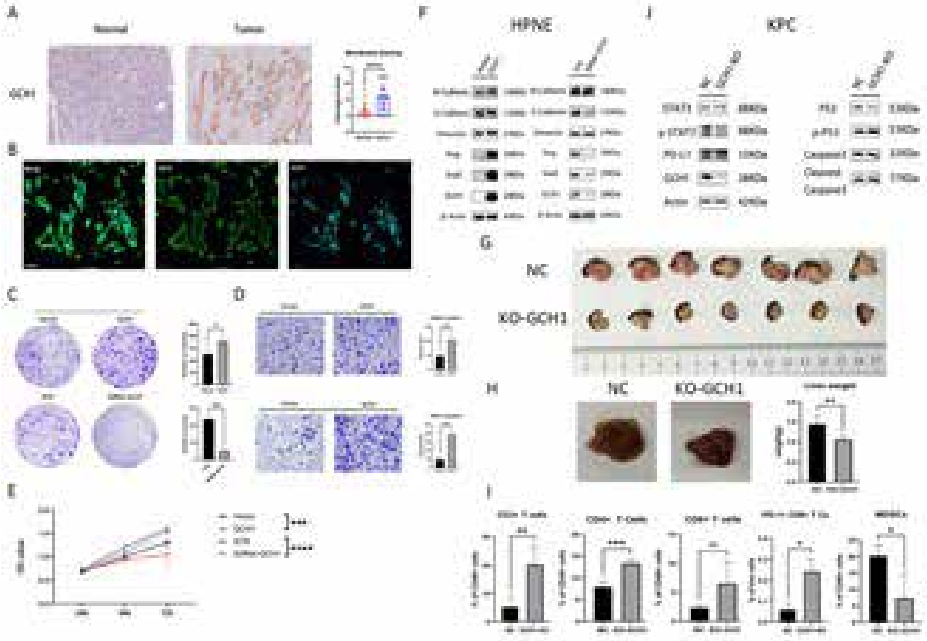

Figure: (A) IHC image for IHC from PDAC samples. (B) mIHC image for IHC and CK19 from PDAC samples. (C) Colony formation assay in GCH1-overexpressed and siRNA-GCH1 treated PANC-1 cells. (D) Transwell invasion& migration assay in GCH1-overexpressed and siRNA-GCH1 treated PANC-1 cells. (E) CCK8 assay in GCH1-overexpressed and siRNA-GCH1 treated PANC-1 cells. (F) Western blot for EMT marker in GCH1-overexpressed HPNE cells. (G) Orthotopic PDAC model and hepatic metastasis model using GCH1 knockout KPC cells. (I) FACS data from orthotopic PDAC model. (J) Western blot for STAT3, P53, PD-L1 and Caspase3 in GCH1 knockout KPC cells

Poster number: 18

GCH1 promotes EMT and PD-L1/PD-1 tumor immune evasion leading PDAC to hepatic metastasis

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Research Rationanle: GTP-cyclohydrolase 1 (GCH1) is the rate-limiting enzyme of tetrahydrobipterin (BH4) synthesis. So far,only limited studies have discussed the role of GCH1 in tumor microenvironment while some of the conclusions contradicted others. In this study, we will try to elucidate the effect of GCH1 besides modulating BH4 synthesis, and aim to clarify its role in immuno-microenvironment in pancreatic cancer and hepatic metastasis.

Methods & Results: IHC and multiplex-IHC were performed in 35 pairs of PDAC (pancreatic ductal adenocarcinoma) samples and the expression of GCH1 was higher in tumor tissues compared to paracancerous tissues (Fig A-B). In vitro experiments including CCK8 assay, colony formation assay, transwell assay supported the hypothesis that GCH1 could increase viability and invasion of pancreatic cancer cells (Fig C-E), while the increased invasion partially resulted from the activation of EMT (epithelial-mesenchymal transition) (Fig F).By constructing the orthotopic injection and hemi-spleen injection models, we also found that GCH1 knock-out could significantly inhibit the tumor growth in orthotopic pancreas and hepatic metastasis using KPC (KrasG12D; P53flox/flox; PDX-1-Cre) cells (Fig G-H). Moreover, GCH1 knock-out resulted in the increased infiltration of T cells and decreased MDSCs in TME (tumor microenvironment) yet the percentage of PD-1+ CD8+T cells was also increased (Fig I). In light of PD-L1 was known as downstream target of JAK2/STAT3 signaling pathway, we performed western blot with GCH1-knockout KPC cells and found the pathway was inhibited and the expression of P53 was also decreased (Fig J).

Conclusion: GCH1 could promote EMT and regulate TME through JAK2/STAT3/P53 pathway, which lead to impaired tumor growth in hepatic metastasis and orthotopic pancreas.

Poster number: 19

Mapping the human bone marrow using spatial transcriptomics in myeloproliferative neoplasia

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Myeloproliferative neoplasia (MPN) is a blood cancer characterised by a well-defined molecular landscape and variable clinical phenotype. A minority of patients will develop bone marrow (BM) fibrosis ('myelofibrosis'), which associated with a poor clinical prognosis. The BM microenvironment underpins the development of fibrosis, however mechanisms that drive this are not fully defined. There is also a pressing need for improved clinical risk stratification in MPN. Emerging spatial transcriptomic (ST) platforms now offer up to subcellular resolution for comprehensive spatial profiling. We systematically and quantitively map the human BM, identifying microenvironmental features that characterise the BM in both health and in patients with MPN.

Method: We used the 10x Xenium platform to perform ST analysis on human archival formalin-fixed paraffin embedded (FFPE) bone marrow trephines (BMTs), including those showing normal/reactive features (n = 5) and BMTs from patients with MPN (n = 25).

Results: We provide a comprehensive spatial map of the human BM across a total of 6,304,447 cells. We identify a peri-adipocytic haematopoietic stem cell (HSC) niche and spatial trajectory of myelopoiesis. We identify expression-based correlates of cellular cytomorphology that define haematopoiesis. We find widespread topological perturbation of nearly all lineages in myelofibrosis and describe microenvironmental immune correlates of BM fibrosis. We identify novel microenvironmental signatures that define the BM in both health and in MPN.

Conclusion: We provide a quantitative map of the human BM, identifying microenvironmental features that underpin BM fibrosis, and spatial feature sets that have potential clinical utility in patients with MPN.

Poster number: 20

Photon-counting Cardiac Computed Tomography for Acute Myocardial Injury Characterisation: Validation Relative to Cardiac Magnetic Resonance Imaging

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Rationale: Characterizing myocardial injury in acute coronary syndromes (ACS) is crucial for diagnosis and prognosis. While cardiac magnetic resonance (CMR) imaging is the gold standard, photon-counting cardiac computed tomography (PCCT) offers ultra-high resolution and spectral capabilities, providing a promising alternative for simultaneous coronary and myocardial assessment. In this study we conduct the first comparative study of PCCT and CMR in characterizing and quantifying acute myocardial injury in ACS.

Methodology: Thirty ST-segment elevation ACS (STEACS) patients underwent same-day PCCT and CMR imaging. We compared microvascular obstruction (MVO), infarct size (IS), extent of injury, and the diagnostic accuracy of late iodine enhancement (LIE) PCCT against late gadolinium enhancement (LGE) CMR to identify prognostically significant acute myocardial injury.

Results: Quantification of infarct size (IS) and microvascular obstruction (MVO) using LIE PCCT showed a strong correlation with LGE CMR measurements, with rho values of 0.97 (95% CI: 0.94–0.99, $p < 0.001$) and 0.95 (95% CI: 0.90–0.98, $p < 0.001$), respectively. LIE PCCT slightly overestimated IS and MVO by 1.02% (LoA: 7.39%) and 0.18% (LoA: 2.27%), respectively, without evidence of systematic bias. Among 480 myocardial segments, PCCT reliably classified transmural extent, achieving excellent agreement in segment viability (Cohen's $\kappa = 0.80$, 95% CI: 0.72–0.86, $p < 0.001$). LIE PCCT accurately identified prognostically significant MVO.

Conclusions: In STEACS, PCCT enables accurate coronary and myocardial imaging, quantifying injury, classifying transmural extent, and identifying prognostically significant MVO. Larger studies are ongoing, but PCCT shows strong potential for improving ACS diagnosis and management.

Poster number: 21

Hyperglycaemia-Induced Trained Immunity in Type 2 Diabetes patients following myocardial infarction

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After myocardial infarction (MI), patients with type 2 diabetes (T2D) have an increased rate of adverse outcomes. Innate immune processes of inflammation likely play an important role(1). We have previously shown how hyperglycaemia induces trained immunity i.e. primes innate immune cells for pro-inflammatory gene expression and function(2). Hence, we hypothesise that following MI, innate immune cells in patients with T2D will display exaggerated and prolonged inflammatory responses and suppression of pathways of inflammation resolution, which are all manifestations of hyperglycaemia-induced trained immunity (HITI).

Methods: In 10 patients with ST-segment-elevation myocardial infarction (5 with T2D and 5 without) blood samples were collected at presentation and at 48 hours and 3 months after MI. Single-cell RNA sequencing was undertaken on peripheral leukocytes.

Results: CD14+ monocytes showed the greatest number of differentially expressed genes. Gene set enrichment analysis of Hallmark pathways identified changes in the response to MI. Compared with patients without T2D, CD14+ monocytes at all 3 time points showed positive enrichment of the Interferon Alpha Response (adj. p-value = 0.04 x 10-2 and 2.5 X 10-4 and 3.9 x 10-2 respectively). In addition, a time-course analysis of the acute response to MI revealed negative enrichment of oxidative phosphorylation in CD14+ monocytes from patients with T2D (adj. p-value = 2.9 x 10-2) but positive enrichment in patients without T2D (adj. p value =2.1 x 10-7).

Conclusions: Our findings suggest that after MI, patients with T2D exhibit altered (i) inflammatory and (ii) metabolic profiles in CD14+ monocytes, characterised by enhanced interferon alpha signalling, a pathway that has been shown to mediate adverse outcomes after MI(3), and impaired oxidative phosphorylation. These changes may account for the adverse prognosis in T2D patients.

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Poster number: 22

Effects of software on myocardial T1 measurements: A 3 T multi-centre single vendor phantom study

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7Siemens Healthcare Limited, Erlangen, Germany

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Research rationale: T1-mapping is an MRI technique used to detect tissue damage in the heart. There is demand for multi-centre studies using T1-mapping, but inter-site differences in values make such studies difficult. Different MRI machine software versions might cause some of these differences.

Methodology: 10+ ShMOLLI T1 maps of phantoms were collected at 12 sites on software version VE11. Reference T1 and T2 maps were collected

in the same scan. An Oxford dataset had previously been used to fit a model that predicts ShMOLLI T1 values based on reference T1 and T2 for the older software version VB17 [1]. The original model was applied to the multicentre data as well as a modified model scaled by a fixed percentage. A suitable scaling value was reached by iteratively calculating the median of percentage deviation (ΔT1) for passing scans.

Results: None of the scans passed the original QC model (figure 1). The QC model was modified to adjust ShMOLLI T1 value predictions by -3.2%, reflecting the overall bias in T1 values on the newer software version. After modified QC 177 out of 235 individual T1 scans fell entirely within the acceptance range (figure 2). All scans lying outside the acceptance range have evidence of problems with acquisition.

Conclusion: This result suggests that it is important to report software version for T1-mapping. The underlying physical causes of the software change deserves further scrutiny.

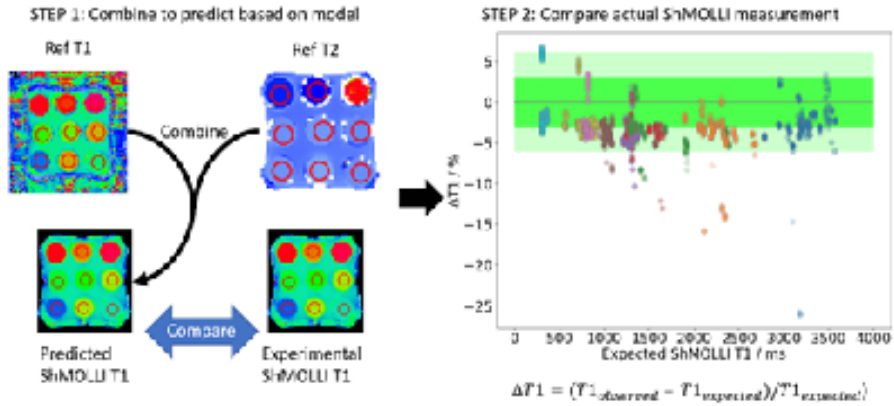

Figure 1 – All data on the new software version is shown on the Bland-Altman style figure with pre-defined acceptable limits in green on the right. Points are coloured by position in the phantom

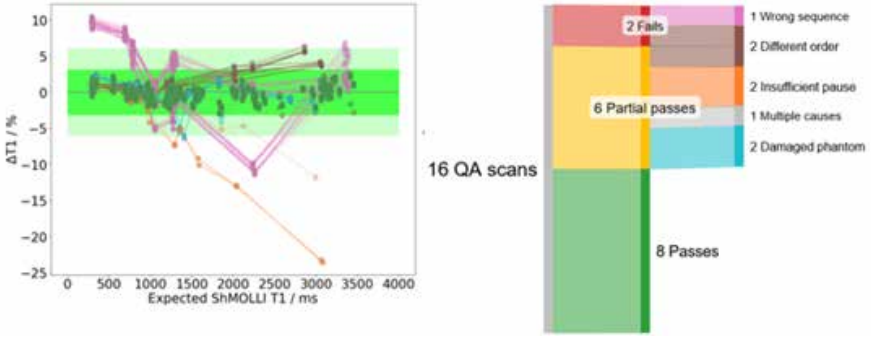

Figure 2 - Results from modified QC. Lines connect points from the same failing scan. Colours correspond to causes for failure

Poster number: 23

The effect of the bone marrow microenvironment in B-lineage acute lymphoblastic leukaemia upon anti-leukaemia T cells

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B-lineage acute lymphoblastic leukaemia (B-ALL) is an aggressive malignancy with historically dismal prognosis in relapsed/refractory cases. Whilst outcomes have improved with genetically-modified, chimeric antigen receptor (CAR) T cells, most patients receiving CAR T cells relapse within two years1. I investigated the effects of B-ALL on the bone marrow stroma, and whether changes in the microenvironment might contribute to relapse after CAR T cell therapy. Using a murine model of B-ALL with the same genetic basis as human disease, I performed experiments using confocal imaging, flow cytometry, single cell mRNA sequencing and functional mouse models. We found that B-ALL induced marked disruption of the architecture of leptin receptor-expressing mesenchymal stromal cells (LepR+ MSCs) (Fig. 1). Single cell transcriptomics revealed that B-ALL remodelled the LepR+ MSC compartment, generating new clusters with an inflamed signature. These inflamed LepR+ MSCs had markedly reduced expression of T cell supportive cytokines, IL-7 and IL-15 (Fig. 2), and increased expression of inhibitory ligands and chemokines. Conditional depletion of IL-7 from LepR+ MSCs in mice receiving CAR T cells resulted in decreased numbers of CAR and endogenous T cells (Fig. 3). This work suggests a contribution of the bone marrow stroma to CAR T cell failure, and sets the scene for further work exploring these mechanisms with a view to identifying modifications to existing treatments.

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1 *Grover P, Veilleux O, Tian L, Sun R, Previtera M, Curran E and Muffly L (2022). Chimeric antigen receptor T-cell therapy in adults with B-cell acute lymphoblastic leukemia. Blood Adv, 6(5), pp. 1608-1618.*

Fig. 1: Representative images of sections from diaphyses of control reporter mouse (left) and leukaemic mouse (right). LepR⁺ MSCs are shown in green; blood vessels are shown in red

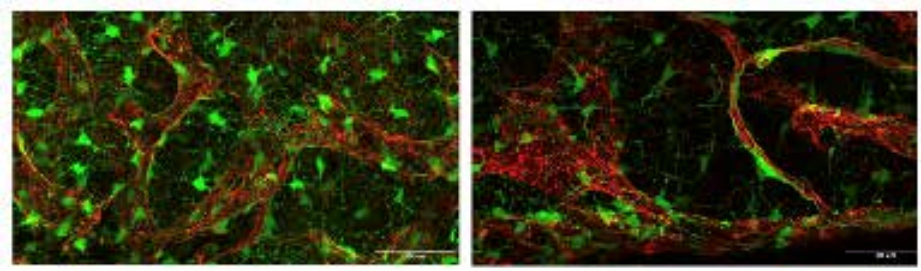


Fig. 2: Relative expression by LepR⁺ MSCs of T cell supportive cytokines in the presence and absence of B-ALL

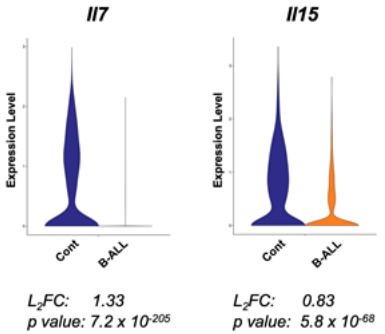
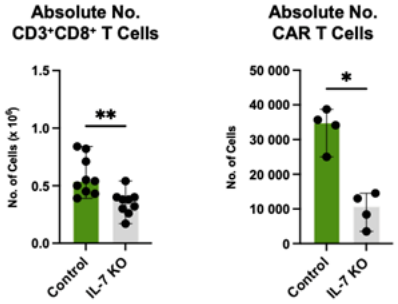


Fig. 3: Bone marrow T cells in Il7 conditional knockout mice vs. control, 8 weeks after infusion of CAR T cells.



Poster number: 24

Generation of Bioengineered Extracellular Vesicles for Immune Cell Targeting

Authors: Charlotte Hooper[1]*, Paul Robinson[1], Rebecca Rooney[1], Dhanu Gupta[2,3], Robin P. Choudhury[1],
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*Postdoctoral Scientist

Affiliations:

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3Department of Laboratory Medicine, Karolinska Institutet, Alfred Nobels allé 8, 141 52 Huddinge, Sweden

Background: Following myocardial infarction (MI), peripheral blood neutrophils and monocytes increase in abundance, with their pro-inflammatory activation correlating with the severity of myocardial injury. While these immune cells also contribute to myocardial repair by infiltrating the injured tissue, current immunomodulatory strategies struggle to suppress excessive inflammation while effectively promoting resolution. Endothelial cell-derived extracellular vesicles expressing vascular cell adhesion molecule 1 (VCAM-1+ EVs) are rapidly released into circulation post-MI, where they mobilise cells from the splenic reserve and induce transcriptional activation prior to recruitment. Neutrophils and monocytes express the VCAM-1 receptor very late antigen-4 (VLA-4), enabling targeted interactions. Here, we develop bioengineered VCAM-1+ EVs as a precision tool for therapeutic immunomodulation.

Methods & Results: Bioengineered EVs were generated by designing fusion constructs incorporating the extracellular domain of VCAM-1 into the abundant EV associated protein CD63, followed by ligation into lentiviral pLVX-Puro plasmids. A FLAG-tag was incorporated to facilitate ectopic expression verification and bioengineered EV selection. To track EV uptake, a non-mammalian microRNA (miR-39-3p) was luminally tethered to CD63. HEK-293 cells were transduced with viral supernatants followed by puromycin selection. Cellular and EV membranes were subsequently fluorescently labelled with lentiviruses encoding PalmtdTomato, enabling visualisation of cellular uptake and live-cell imaging. Total EVs were isolated via ultracentrifugation, characterised using Nanoparticle Tracking Analysis and Western blotting, and enriched using anti-FLAG antibody-conjugated microspheres.

Conclusions: We have developed a bioengineering platform to generate VCAM-1+ EVs, providing a novel tool for targeted immunomodulation. Ongoing studies will administer these EVs in a mouse model of MI to investigate their immunoregulatory effects on neutrophils and monocytes.

Poster number: 25

Loss of histone H4 lysine 16 acetylation in macrophages following pro-inflammatory stimulation is required for activation of inflammation

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*postdoc

Rationale: Myeloid cells are crucial effectors of the innate immune response providing host-defence against pathogens and tissue damage. Macrophage phenotype is tightly regulated by the differential binding of transcription factors, in response to internal and external signals, which establish and modify the epigenetic landscape including chromatin accessibility, histone modifications, and DNA methylation. In the present study, we systematically interrogate global histone modifications following pro-inflammatory 'M1' activation of macrophages using quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methodology: Monocytes from healthy donors (n=3) and bone marrow cells from wild-type mice (n=3) were isolated, differentiated into macrophages and stimulated with LPS and IFN-γ. Histones were acid-extracted, propionylated and digested using trypsin and measured by LC-MS/MS.

Results: LC-MS/MS revealed a significant reduction in global H4K16ac (by 5%; $P<0.01$) following pro-inflammatory macrophage stimulation, which was conserved across species ($P\leq0.01$). This reduction was validated by western blot in an independent donor. Reduction of H4K16ac levels with MG149, an inhibitor with efficacy against MOF, responsible for bulk of H4K16ac, prior to pro-inflammatory stimulation exacerbated the inflammatory response to LPS and IFN-γ ($P<0.05$), and conversely, re-adjusting acetylation levels (including H4K16ac) using HDAC inhibitors inhibited the inflammatory response to LPS and IFN-γ ($P\leq0.01$).

Conclusions: LC-MS/MS identified significant changes in the global abundance of histone modifications, specifically H4K16ac, following pro-inflammatory activation of macrophages. Given the crucial role of epigenetics in the activation and pro-inflammatory responses of innate immune cells, in combination with the fact that dysregulated macrophage phenotype is a major driver of inflammation-related diseases such as atherosclerosis, the development of drugs that target specific epigenetic modifications could lead to a novel era of therapeutic intervention in inflammation-related conditions.

Poster number: 26

Macrophage rhythms: investigating how clock machinery directs circadian behaviour

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Much of physiology is under circadian regulation: there is 24-hour time-keeping, cell-autonomous machinery in nearly every cell. Inflammation is often rhythmic. This rhythmicity is a hallmark of inflammatory diseases like rheumatoid arthritis. Macrophages are key drivers of inflammation, and they are highly rhythmic cells. Rhythms in gene expression may be driven by external cues such as nutrient cycles from feeding/fasting, or may be internally-derived and driven by the core-clock machinery. To investigate this, we first identified the rhythmicity resulting only from the core-clock machinery. We compared peritoneal macrophages from both wild-type and Cre-dependent Bmal1-knockout mice at two timepoints. Bmal1 is an essential core-clock gene, crucial for rhythmicity. We identified clock-dependent changes to transcription, and by stimulating macrophages with LPS injection we confirmed that the inflammatory response alters the rhythmic transcriptome. The transcriptomes show an augmented inflammatory response in Bmal1-knockout mice compared to wild-type. We also found that Bmal1 knockout leads to distinct transcriptomic differences between male and female mice.

However, we do not yet understand how exactly the core clock machinery generates rhythms in the target genes in macrophages. We used the transcriptomes from two time-points to identify a set of genes responsive to LPS in a time-of-day dependent manner. To understand how the core clock drives this time-of-day difference in the expression of these genes, we have used ATAC-seq to map chromatin accessibility and can relate this to enhancer activity using epigenomic markers and transcription factor cistromes. Understanding how circadian rhythms are generated in macrophages could provide insight into how to manipulate them for therapeutic treatments, as well as how to better manage immunological diseases.

Poster number: 27

Towards a directed evolution approach to improve lentiviral vector targeting

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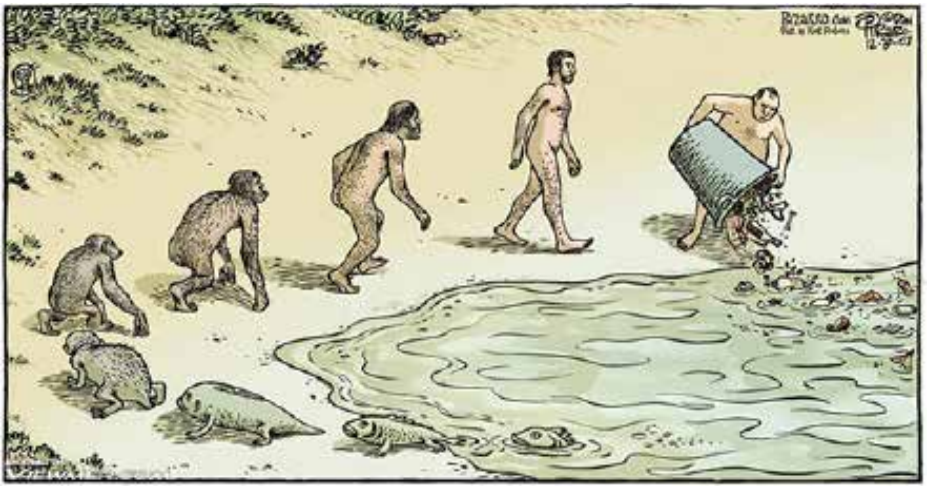
Recombinant lentiviral vectors (rLVs) hold promise for treating monogenic disorders due to their ability to transduce a broad spectrum of cell types, including non-dividing cells. However, the broad tropism of the commonly used VSV-G pseudotype can limit targeting specificity. This highlights the need for vectors with improved cell-type specificity to reduce off-target effects and expand applications beyond ex vivo gene delivery. Here, we describe a directed evolution platform to engineer rLV pseudotypes derived from VSV-G and the lung-targeting F/HN pseudotype (based on Sendai virus glycoproteins). These pseudotypes are expected to acquire distinct phenotypes, including altered binding and/or targeting properties.

Our two-round viral production strategy uses a vector genome with an intact U3 region that conditionally produces viral RNA via Tat, enabling efficient vector mobilisation. Mobilisable rLVs pseudotyped with VSV-G and encoding F, HN, VSV-G, or EGFP were generated using HEK293T-tetR cells. Functional titres achieved were 8.82E+06, 1.33E+07, 5.71E+07, and 2.07E+08 TU/mL, respectively.

In the second round, VSV-G pseudotyped particles transduced cells at MOI 1. Measurable titres for VSV-G were 1.31E+05 TU/mL, while F and HN pseudotypes required higher MOIs to achieve titres of 4.9E+04 and 1.24E+05TU/mL. These data demonstrate important proof-of-concept for the feasibility of the approach with the VSV-G group and shows that rLVs encoding their own viral RNA and pseudotype glycoprotein can be efficiently produced even at low MOI. Future iterations will focus on bar-coded libraries of F and HN variants to identify pseudotypes with improved transduction efficiency and cell-specificity.

Figure 1. Natural evolution is a slow and unpredictable process. No virus will ever naturally evolve to deliver therapeutic genes to clinically relevant cells. Directed Evolution allow us to subject a population of variants, in this case sequences, to selective pressures ensuring the survival and reproduction of those with the advantageous traits.



Poster number: 28

Network-based discovery of genes linked to rare developmental disorders

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Affiliations: 1 Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, UK, 2 Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, UK, 3 Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, UK

Rare disorders affect approximately 3.5 million individuals in the UK, with many patients experiencing prolonged diagnostic journeys that can sometimes span decades. Previous research has linked SATB1 and SATB2 to neurodevelopmental disorders, suggesting that analyzing their interaction networks could uncover novel candidate genes. This study aims to identify new genes implicated in rare neurodevelopmental disorders through a network-based approach and analysis of large genomic datasets.

Network analysis was performed using BIOGRID, STRING, and ClueGo to identify SATB1 and SATB2 interactors. Whole exome sequencing data from 33,264 individuals in the Deciphering Developmental Disorders (DDD) study were analyzed looking for rare variants in the SATB interactors. Variants were annotated using ANNOVAR and filtered upon ACMG guidelines. The patient cohort was expanded using data from the 100,000 Genomes Project and NHS GMS databases.

A total of 87 interactors of SATB1 and SATB2 were identified, of which 22 (25.3%), including SATB1 and SATB2, are recognised to be involved in neurodevelopmental syndromes. Bioinformatic analysis of the DDD dataset uncovered 209 pathogenic or likely pathogenic variants in genes that have not yet been implicated in rare disorders. Among these, patients with potentially deleterious variants in HDAC1 and HDAC2 have been identified who present with global developmental delay, intellectual disability, hypotonia and additional phenotypic abnormalities. These findings provide a foundation for developing functional studies to evaluate the pathogenicity of these variants.

Poster number: 29

The Soluble Isoform of the Type I Interferon Receptor is a Negative Regulator of Antiviral Gene Expression

Authors: Alexander Redmond (DPhil Student), Anne Bridgeman, Elizabeth Horton, Tanja Davis, Jan Rehwinkel

Affiliations: Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK

Type I interferons (IFNs) are a critical component of the innate immune system, contributing towards antiviral defence, cancer surveillance, immunoregulation, and autoimmunity. Whilst the transmembrane isoform of the type I IFN receptor, IFNAR2, is well-studied, the role of its soluble isoform remains poorly understood. This project aims to investigate whether soluble IFNAR2 acts as an agonist or antagonist of the type I IFN response.

We generated a soluble Ifnar2-/- mouse model, ensuring the transmembrane isoform of IFNAR2 was preserved. Mice or whole blood were stimulated with type I IFN, and downstream IFN-stimulated gene and cytokine responses were analysed. In vitro cytopathic effect assays were performed using virus-infected Ifnar2-/- mouse embryonic fibroblasts (MEFs). Finally, we crossed soluble Ifnar2-/- mice with the Adar1mZa/mZa strain, which exhibits a low, constitutively active type I IFN signature, to determine whether the absence of sIFNAR2 influenced this phenotype.

The absence of soluble IFNAR2 increased IFN-stimulated gene expression and CXCL10 cytokine production following in vivo and ex vivo type I IFN stimulation, implicating soluble IFNAR2 as a negative regulator. In vitro assays revealed reduced cytopathic effects of HSV-1 in the absence of soluble IFNAR2, with diminished infection and killing potential observed. Furthermore, crossing soluble Ifnar2-/- and Adar1mZa/mZa mice exacerbated the phenotype of baseline IFN-stimulated gene expression.

Collectively, these findings show that soluble IFNAR2 functions as a negative regulator of the type I IFN response, potentially exerting an antagonistic effect systemically. Understanding its function could provide insights into developing targeted therapies for conditions involving dysregulated IFN activity.

Poster number: 30

X-NET and Beyond: How to INTEGRATE Data Scientists in Biomedical Research

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Diverse teams are the key to innovation when tackling the complex challenges faced in biomedical research with interdisciplinary and 'team science' being recently championed by UK research funders. However, researchers from interdisciplinary backgrounds, such as those with computational or mathematical backgrounds often face barriers in their careers which can hinder effective collaboration and integration in UK biomedical research. The MRC-funded cross-disciplinary network X-NET was designed to identify these challenges and create a set of recommendations to UKRI on how to tackle them. Here, we present key findings from X-NET and outline strategies for assessment of effective interventions as part of the INTEGRATE project.

Opinions of interdisciplinary researchers at different career stages across the UK were collected by survey (n=76) and a focused workshop (n=30). Participants were asked to reflect on personal and institutional/cultural barriers to interdisciplinary research and consider specific actions to overcome them. Further workshops were held with stakeholders from industry (n=12) and PPI groups (n=14) alongside interviews with key opinion leaders to inform recommendations to improve interdisciplinary research.

X-NET reported 13 recommendations to UKRI which focused on 3 key themes: increasing mobility, nurturing environments, and equitable evaluation. In addition, tools, including the Wheel of Privilege were developed to facilitate discussions about how barriers can be tackled. INTEGRATE expands this work as part of a MRC Biomedical Data Science Leadership Award, identifying 'best practices' for supporting interdisciplinary researchers within research organisations and assessing the ability of interventions to increase integration of data scientists with the biomedical research community.

Poster number: 31

Evasins bind chemokines through conformationally plastic interfaces to overcome disease-specific chemokine network robustness

Authors: Charlie J. Clark1 (DPhil Student), Megan Payne1, Serena Vales1, Finnian Gavin1, Soumyanetra Chandra1, Gintare Smagurauskaite1, Garrett M. Morris2 & Shoumo Bhattacharya1

Affiliations: 1 – Centre for Human Genetics & RDM Cardiovascular Medicine, University of Oxford, University of Oxford, 2 – Department of Statistics, University of Oxford.

Rationale: Functional interactions between chemokines and receptors drive immune cell trafficking and underlies all inflammatory disease. However, targeting individual chemokines or their receptors has hitherto failed as a therapeutic approach. The efficacy of multi-chemokine binding tick evasin proteins in models of vascular, skin, liver and gut inflammation, suggests that natural selection has arrived at a different approach. Here, we aimed to understand why chemokine signalling networks in disease are a difficult therapeutic target and how tick evasins overcome them.

Methodology: We analysed single-cell RNA sequencing data from atherosclerosis, metabolic dysfunction-associated steato-hepatitis, psoriasis and Crohn's disease to identify expressed chemokines, receptors and immune cell classes. We constructed networks using this information and chemokine-receptor interactions identified from IUPHAR. We analysed the robustness of these networks using secondary extinction analysis. We performed structural analysis of chemokine:evasin, chemokine:receptor and chemokine:chemokine complexes using AlphaFold 3.

Results: We identified tripartite chemokine networks in 4 human inflammatory diseases. These networks are highly robust to chemokine or receptor deletion. Network robustness is significantly reduced by evasins. Structural analyses using AlphaFold 3 suggest that evasins bind chemokines through conformationally plastic interfaces to sterically hinder functional chemokine interactions with receptors and with other chemokines.

Conclusions: Our findings demonstrate the existence of robust chemokine signalling networks in disease and explains failure of current therapeutic approaches targeting single chemokines or receptors. They show that tick evasins decrease network robustness by inhibiting multiple functional chemokine interactions, explaining their efficacy in inflammatory disease models. We suggest that anti-inflammatory therapeutics will need to decrease chemokine network robustness in disease.

Poster number: 32

Circadian Clock and Macrophage Cytoskeleton Function

Authors: Siyu Chen (DPhil student), Hua Pu, Fraydoon Rastinejad, Laura Bailey, Nic Ciccone, David Ray

Affiliations: Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, UK

The circadian clock generates daily physiological rhythmic oscillations with a period length of approximately 24 hours. These are aligned to the external light-dark environment resulting from the actions of Zeitgebers. Macrophages, as central components of the innate immune system, are considered key orchestrators of circadian, immune, and metabolic functions and are attractive therapeutic targets. We investigated how BMAL1 (Basic Helix-Loop-Helix ARNT Like 1), a core circadian clock component, affects macrophage characteristics and functions. We first tested the effect of BMAL1 on macrophage morphology and motility using BMDMs (bone marrow-derived macrophages) as a general macrophage model. Wild-type and Bmal1-knockout BMDMs were treated with vehicle or a novel small-molecule BMAL1 ligand named CCM (core circadian modulator), under basal conditions or following stimulation with LPS, a potent macrophage activator. Then BMDM actin organisation was measured with confocal microscopy followed by 3D reconstruction, and motility was recorded with live cell imaging. Under basal states, both Bmal1 deletion and CCM caused BMDM cell area to decrease and height to increase, indicating that the cells were less adherent, and more rounded, suggesting higher motility. Interestingly, CCM weakened the effect of LPS on BMDM morphology. BMDM motility was also affected by such genetic and pharmacological BMAL1-targeting approaches. Together, these findings indicate that BMAL1 is potentially a key component linking timing and macrophage cytoskeleton organisation, therefore regulating the fundamental clearance functions of macrophages. As macrophages are highly heterogeneous and plastic, we will further investigate the role of BMAL1 in Kupffer cells, liver-resident macrophages, in health and inflammatory diseases.

Poster number: 33

Using Induced Pluripotent Stem Cells to Model Lung Surfactant Deficiency

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Affiliations: Gene Medicine Group, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine

To advance gene therapy for lung diseases, we have developed a physiologically relevant model of alveolar function using induced pluripotent stem cells (iPSCs). iPSCs provide a renewable source of alveolar epithelial type II (ATII) cells, which are essential for pulmonary surfactant production and alveolar homeostasis. We have generated three ABCA3 knockout (KO) iPSC lines, confirmed by Sanger sequencing, which we are differentiating into ATII cells to model ABCA3 surfactant protein deficiency, a severe recessive disorder causing neonatal respiratory distress.

Our differentiation approach follows a directed protocol that mimics in vivo lung development, allowing for the generation of self-renewing alveolospheres with ATII-like characteristics (PMID: 31732721). Notably, differentiation from iPSCs to ATII cells enables the emergence of phenotypes previously unobserved in immortalized cell line models, potentially revealing novel aspects of ABCA3 dysfunction. These iPSC-derived ATII cells are cultured at the air-liquid interface (ALI) under conditions that support polarization and surfactant metabolism. Functional assessments include lipid dysregulation, transepithelial electrical resistance, and characterization of surfactant storage granules.

Our iPSC-derived model provides a scalable and renewable system that surpasses primary ATII cells in longevity and enables precise genetic modifications. In the absence of a robust murine model for ABCA3 deficiency, this platform offers an opportunity to study surfactant dysfunction at a molecular level and to evaluate genetic therapies for surfactant protein disorders.

Poster number: 34

Localised GLP-1 receptor pre-internalization directs pancreatic alpha cell to beta cell communication

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Affiliations

1Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), Churchill Hospital, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

2Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin, Germany.

Pancreatic cells modulate beta cell function in a paracrine manner through the release of glucagon. Failure of alpha cell › beta cell communication leads to impaired insulin secretion and hyperglycaemia. However, the detailed molecular architecture underlying alpha cell › beta cell regulation remains poorly characterised. Here, we show that glucagon-like peptide-1 receptor (GLP1R) is enriched as nanodomains on beta cell membranes in close contact with alpha cells, in keeping with increased single-molecule transcript expression. Beta cells located next to alpha cells directly sense glucagon release at low glucose by pre-internalising GLP1R. Pre-internalised GLP1R primes beta cells to respond to high glucose with early rises in cytosolic Ca2+ signals, which are propagated across the islet. Beta cells adjacent to alpha cells are more secretory than beta cells next to other beta cells. Localised GLP1R signalling occurs in vitro and in vivo, is operative in the postprandial state, and GLP1R contacts decrease between beta cells and alpha cells with advancing age and high fat diet. Thus, we detail a regulated pathway through which glucagon modulates insulin release. More broadly, we provide a framework for how G protein-coupled receptors and promiscuous signalling fine-tune intercellular communication in complex tissue.

Poster number: 35

CRISPR-Cas9 based interrogation of the T cell kinase and phosphatase network reveals novel mediators of inhibitory signalling through PD-1 and BTLA

Authors: Joseph Clarke, Toby Whitehead, Simon J. Davis, Sumana Sharma.

Affiliations: Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, OX3 9DS, UK

Clinical administration of blocking antibodies to immune checkpoint receptors is now a well-established therapeutic intervention for the treatment of many malignancies. In particular, blockade of the PD-1 signalling axis has revolutionised the field of cancer immunotherapy and is now considered a first line of treatment for numerous cancers. However, there is still an unmet clinical need to better understand signalling through these immune checkpoint inhibitors given that the vast majority of patients that receive this therapy are non-responders and hence do not clear their tumours. Therefore, better understanding of signalling pathways initiated downstream of checkpoint receptor ligation may reveal new therapeutic targets and provide the rationale for the designing of new immunotherapies. Here, we have undertaken a CRISPR-Cas9 approach to study the signalling pathways utilised by two key checkpoint receptors, namely PD-1 and BTLA. Using this methodology, we have individually deleted each of the 750+ kinases and phosphatases, as well as key adaptor molecules, that T cells can potentially express, providing to our knowledge the first systematic interrogation of the complete kinase – phosphatase network utilised by T cells in the context of both activation through the TCR, and inhibitory signalling through checkpoint receptors PD-1 and BTLA. This large scale, systematic approach, identifies both known and novel mediators of T cell inhibitory signalling pathways. Our screen reliably identifies PTPN11, the gene encoding SHP-2, as a key mediator of signalling through PD-1 and BTLA, in line with the current thoughts of the T cell inhibition field. Additionally, we identify other key signalling molecules in classical T cell activation pathways (such as proteins involved in both PI3K and calcium signalling pathways) as critical for mediating T cell inhibition, as well revealing a role for a novel transcriptional repressor in T cell inhibition, that can be targeted for improving existing immunotherapies as well as designing new CAR-T cell products.

Poster number: 36

Modifying TTN gene regulation and expression in human iPSC-CMs to investigate mechanisms and phenotypes of TTN-associated dilated cardiomyopathy

Authors: Rosemary Kirk1, Alexander Sparrow1, Charlotte Hooper1, Violetta Steeples1, Christopher Toepfer1, Hugh Watkins1

Affiliations: 1Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, United Kingdom

Rationale: Heterozygous truncating variants in TTN are the leading genetic cause of familial dilated cardiomyopathy (DCM). However, the mechanisms by which these mutations cause disease are incompletely understood, with possible roles for both haploinsufficiency and dominant negative effects. There is also much unknown about the regulation of TTN expression. One possible regulator that warrants exploration is the presence of upstream ATGs (uATGs) in TTN's 5'-untranslated region (5'UTR). Such uATGs typically repress translation and can be removed or masked to upregulate gene expression.

Targeting gene expression regulators in human-induced stem cell-derived cardiomyocyte (hiPSC-CM) models of TTN-associated DCM will help clarify TTN's regulatory mechanisms. Further, it will help to untangle relative roles of haploinsufficiency and dominant negative mechanisms in causing disease by seeing how such alterations affect disease phenotype. Addressing these questions is critical to developing genetic therapies for TTN-associated DCM.

Methodology: We used CRISPR-Cas9 gene-editing to generate hiPSC lines with known disease-causing heterozygous truncating mutations in TTN. hiPSCs were differentiated into hiPSC-CMs for phenotyping including measuring titin protein levels and functional read-outs. Cell lines underwent further CRISPR-Cas9 gene-editing to modify suspected regulatory regions of TTN's 5'-UTR and further phenotyping was conducted on the generated hiPSC-CMs.

Results: hiPSC lines with heterozygous truncating mutations in TTN were generated. Differentiated hiPSC-CMs displayed reduced full-length titin protein expression, truncated titin protein, and functional abnormalities. Editing of TTN's 5'UTR region to remove uATGs unexpectedly resulted in significant downregulation of titin protein in hiPSC-CMs compared to unedited controls.

Conclusions: Editing TTN's 5'UTR resulted in unexpected effects on protein expression, suggesting unique regulatory mechanisms for this gene. Further interrogation of these findings will provide valuable insights into TTN regulation, thereby aiding in designing genetic therapies for TTN-associated DCM.

Poster number: 37

Defining molecular interactions between human CD4+follicular helper T cells and CD8+ T cells

Authors: Andrew McIntyre1 (Postdoc), Persephone Borrow2 and Ronjon Chakraverty1

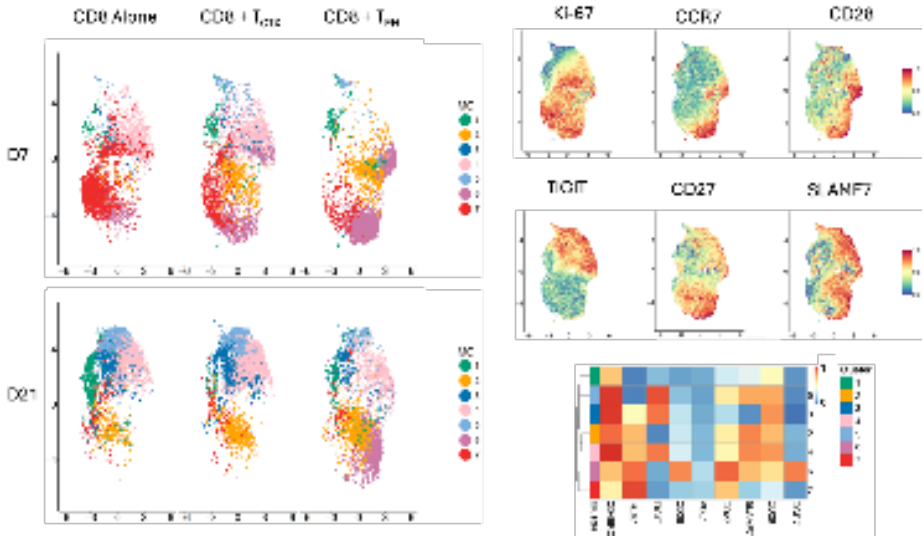
Affiliations: 1 MRC Weatherall Institute of Molecular Medicine, University of Oxford, 2Centre for Immuno-Oncology, Nuffield Department of Clinical Medicine, University of Oxford.

The success of adoptive immunotherapy with anti-cancer T cells depends upon their persistence in vivo and is linked to the transfer of multipotent, early-stage memory T cell populations [1]. We have recently identified that early CD4+ T cell infusion following allogeneic haematopoietic stem cell transplantation in human patients leads to expansion of T follicular helper (TFH)-like cells with features of central memory cells, while antagonising the clonal expansion and differentiation of late-stage CD8+ and CD4+ cytotoxic T cells (TCTX). We are investigating the potential for direct interactions between human CD4+ TFH and CD8+ T cells, with the aim of identifying molecular pathways which could be incorporated in therapeutic T cell manufacturing.

We have compared CD4+ TFH and CD4 TCTX isolated from blood for their effects upon autologous CD8+ T cell differentiation using a model of repetitive stimulation in vitro and a combination of flow cytometric, transcriptomic and functional analyses. Whereas helpless CD8+ T cells or CD8+ T cells cultured with CD4+ TCTX underwent terminal differentiation, CD4+ TFH cells promoted CD8+ T cellular states linked to early memory-like differentiation and proliferative fitness, with no loss to cytotoxic function. While these effects were partially dependent upon the canonical TFH cytokine IL-21, we are now pursuing a determination of the broad range of molecular interactions involved using scRNAseq interactome analyses and proteomic screening of cell culture supernatants with the aim of systematically screening candidate pathways. This research will help to define new methods for manufacturing of therapeutic CD8+ T cells with the goal of optimising their function and persistence.

Krishna S, Lowery FJ, Copeland AR, et al. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. Science. 2020;370(6522):1328-1334. doi:10.1126/science.abb9847

Fig 1- Phenograph clustering of CD8⁺ T cells at d7 and d21 following polyclonal stimulation shows retention of less-differentiated cell states with CD4⁺ T_{FH} help vs no help or CD4⁺ T_{CTX} help



Poster number: 38

Automated Quantification and Spatial Characterisation of Plasma Cell Neoplasia in Bone Marrow Trephine Biopsies

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Purpose of the study: Plasma cell myeloma is an important yet largely incurable blood cancer. The spectrum of plasma cell (PC) disorders ranges from Monoclonal Gammopathy of Unknown Significance (MGUS) through Smouldering Myeloma (SMM) to symptomatic Multiple Myeloma (MM). Complex criteria delineate the boundaries between these entities, but among these the PC burden is a key feature, which alone can shift the diagnosis from MGUS/SMM to MM, thus critically affecting treatment decisions. Unfortunately, biopsy quantification of PCs is known to be unreliable. In response to this, a number of automatic quantification methods have been developed, but none are used routinely. Additionally, patterns of spatial PC distribution are known to be associated with prognosis, but these results have been largely overlooked and have yet to enter into diagnostic practice.

Methods: A dataset of digital images of MGUS/SMM/MM cases has been curated (n=100), including a routine immunohistochemical (IHC) panel (Mum1, CD56, Kappa and Lambda light chains). An automated PC quantification pipeline was developed and used to quantify plasma cells with good performance. Following identification of PCs, the spatial patterns of PC infiltration were analysed using spatial statistical methods, showing a range of spatial patterns at the same level of plasma cell infiltration, promising to open a novel biomarker in assessment of myeloma.

Summary of results: An automated image analysis pipeline for accurate PC characterisation has been developed. The proposed automated method yields comparable results to existing approaches. In addition, the present work offers new insights on spatial patterns of PC infiltration, which constitute a previously overlooked, but potentially important biomarker of PC neoplasia.

Poster number: 39

Straightforward MRI Reconstruction with B0 Correction and Coil Sensitivity Encoding Using Direct Pseudoinversion of the Encoding Matrix (Pinv-Recon)

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MRI reconstruction traditionally relies on the Fast Fourier Transform (FFT) requiring additional steps, such as gridding for non-Cartesian trajectories, and separate corrections for B0 field inhomogeneity and coil sensitivity. Whilst reconstruction using the Moore-Penrose pseudoinverse of an encoding matrix is conventionally considered computationally prohibitive, we demonstrate its feasibility with modern day computational infrastructure, emphasize its applicability to small-to-medium sized images, and highlight its advantages for straightforward inclusion of B0 correction and coil sensitivity encoding. We evaluated this reconstruction method, which we refer to as Pinv-Recon, using both numerical simulations and experimental data.

B0 correction was tested on a Shepp-Logan phantom, a structural phantom, and brain data acquired using spiral trajectories on a 3T GE Premier scanner (Figure 1a). B0 maps were acquired using varied echo time acquisitions of a short readout spiral, and B0 distorted data were acquired by lengthening the readout duration. B0 correction using Pinv-Recon was compared with conventional Multifrequency Interpolation (MFI). In the Shepp-Logan simulations and structural phantom data, Pinv-Recon achieved 0.01 (73%) and 0.001 (63%) lower mean squared error respectively compared to reference images with no B0 distortion. In brain data, though no reference image was available, Pinv-Recon demonstrated improved deblurring.

Coil sensitivity encoding was evaluated using hyperpolarized carbon-13 brain data acquired with an 8-channel head coil (Figure 1b). Pinv-Recon with coil sensitivity encoding enhanced Signal to Noise ratio (SNR) by 70% averaged over time compared to root-sum-of-squares coil combination. The encoding matrix inversion took 0.38 seconds.

This work demonstrates that direct pseudoinversion of the encoding matrix is not only feasible but offers advantages over traditional reconstruction methods, including straightforward implementation, inherent regularization, and improved image quality. Beyond B0 correction and coil sensitivity encoding, it could be extended to other encoding mechanisms such as

chemical shift and gradient non-linearity corrections.

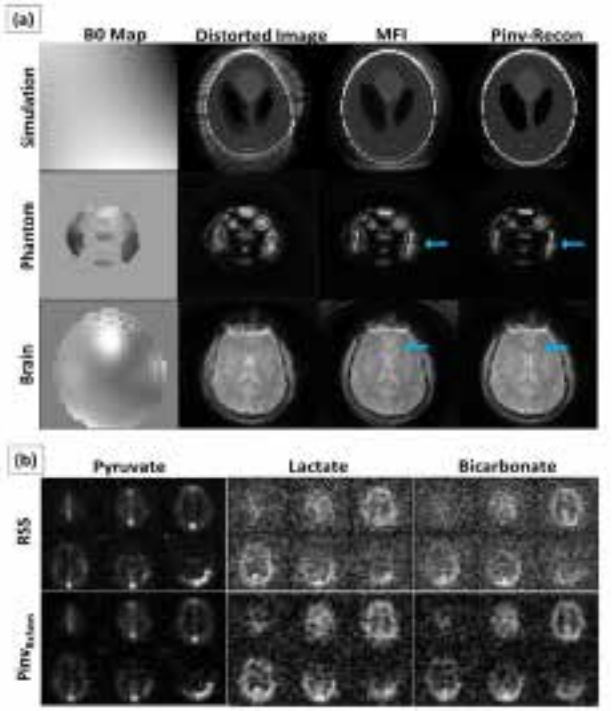

Figure 1 (a) Shepp-Logan simulations, Structural Phantom acquisitions, and in vivo brain acquisitions comparing correction of B_0 distortion using Pinv-Recon versus MFI (b) Pyruvate, Lactate and Bicarbonate images using root-sum-of-squares coil combination versus coil sensitivity encoding in Pinv-Recon.

Poster number: 40

Unmasking Supervillin: Functional Insights in Hypertrophic Cardiomyopathy using iPSC-derived Cardiomyocytes

Authors: Yifan Joshua Li (DPhil Student), Yiangos Psaras, Violetta Steeples, Charlotte Hooper, Alexander Sparrow, Paul Robinson, Hugh Watkins, Christopher Toepfer

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Introduction: Hypertrophic cardiomyopathy (HCM) is a prevalent hereditary cardiac disorder affecting 1 in 500 individuals, with severe clinical implications. Despite genetic advances, nearly 40% of patients remain gene-panel negative. A recent GWAS study1 has implicated truncating variants in SVIL, encoding the actin-binding Z-disc protein Supervillin, as a novel HCM disease gene. This study investigates the functional consequences of SVIL truncation using iPSC-derived cardiomyocytes (iPSC-CMs).

Methods: An isogenic iPSC model (WT, heterozygous, and homozygous SVIL p.Gln255* truncation) was generated using CRISPR-Cas9. Engineered lines were validated for pluripotency, genomic stability, and off-target effects. Differentiated iPSC-CMs were characterized by gene and protein expression, calcium handling (R-GECO), and contractility (GFP-α-actinin) assays. Myosin conformation states were analysed using MANT-ATP assays. The therapeutic potential of Mavacamten was assessed.

Results: SVIL expression analyses confirmed haploinsufficiency in heterozygous and homozygous lines. Functional assays revealed prolonged calcium transients (Toff and Tau) and reduced relaxation velocity, indicative of diastolic dysfunction. Contractility measurements demonstrated shortened diastolic sarcomere length and reduced sarcomere fractional shortening, suggestive of both diastolic and systolic impairment. MANT-ATP analysis revealed a decrease in SRX and an increase in DRX states, implicating altered myosin energetics. Acute Mavacamten treatment ameliorated diastolic dysfunction but exacerbated systolic impairment, suggesting limited therapeutic utility.

Conclusions: This study establishes SVIL truncation as a novel pathogenic HCM variant causing diastolic and systolic dysfunction via haploinsufficiency. Findings suggest Mavacamten may not be suitable for SVIL-related HCM. Ongoing work includes metabolic profiling, bulk proteomics, and engineered heart tissue models to further understanding and refine therapeutic strategies.

Tadros, Rafik, et al. “Large scale genome-wide association analyses identify novel genetic loci and mechanisms in hypertrophic cardiomyopathy.” medRxiv (2023).

Poster number: 41

B-type Natriuretic Peptide Reverses Vascular Endothelial Insulin Resistance and Ameliorates Dysregulated Redox Signalling when Administered Alongside Insulin in Human Atherosclerosis

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Aim: Atherosclerosis remains a leading complication of diabetes mellitus. Physiologically, in endothelial cells, insulin stimulates activation of endothelial nitric oxide (NO) synthase (eNOS), producing the vasorelaxant, anti-inflammatory, and atheroprotective NO. Patients with atherosclerosis exhibit vascular endothelial insulin resistance regardless of diabetic status. When atherosclerosis patients are given insulin, a pathological signalling cascade activates NADPH oxidase (NOX) and uncouples eNOS, resulting in vasoconstriction, inflammation, oxidative stress, and atherogenesis. Hence, diabetic patients on aggressive insulin therapy have poor cardiovascular outcomes. Dipeptidyl-peptidase-IV (DPPIV) inhibitors ameliorate this phenotype ex vivo but do not confer prognostic cardiovascular benefits clinically. B-type natriuretic peptide (BNP) is a DPPIV substrate that may modulate endothelial insulin and redox signalling.

Methods: Samples of plasma, and intact internal mammary artery (IMA) and saphenous vein (SV) were collected from 128 adults undergoing coronary bypass surgery. Plasma BNP and insulin were measured by ELISA. Sequential rings of vessels were incubated in four conditions (control, insulin, BNP, insulin & BNP). Baseline, NOX-derived, VAS2870-inhibitable, and LNAME-delta superoxide production were measured in IMAs through chemiluminescent luminometry. Endothelial function was quantified in SVs with organ bath vasomotor studies assessing endothelium-dependent relaxation with

acetylcholine and bradykinin.

Results: In patients with low plasma BNP, increased endogenous insulin levels are significantly associated with increased IMA NOX-derived superoxide production ($p=0.034$). This effect is abolished in patients with BNP excess ($p=0.50$). Insulin significantly increases IMA resting ($p<0.0001$), NOX-derived($p=0.0016$), and VAS2870-inhibitable ($p=0.0005$) superoxide production and uncouples eNOS NO production ($p=0.027$), whilst BNP combined with insulin restores these to normal levels. BNP with and without insulin recouples eNOS NO production ($p=0.037$). Insulin significantly reduces endothelium-dependent vasorelaxation ($p=0.01$) whilst BNP with insulin significantly increases endothelium-dependent vasorelaxation ($p=0.04$).

Conclusion: BNP resensitises the vascular endothelium to insulin and ameliorates pathological redox signalling when co-administered with insulin. BNP may have a role as a future therapeutic in preventing and treating atherosclerosis, especially with comorbid diabetes.

Poster number: 42

Unveiling the role of cardiac monocyte/macrophages in hypertrophic cardiomyopathy: phenotypic profiling and functional insights

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Myocardial fibrosis, a key feature of HCM, contributes to adverse remodelling and disease progression. While genetic mutations are primary drivers, immune cells also play a crucial role. Our earlier findings show that lymphocyte-deficient Actc1E99K mice experience worsened remodelling, while regulatory T cells (Tregs) help mitigate fibrosis. This suggests that the immune cells regulated by Tregs may drive adverse remodelling in HCM. To investigate further, we examined the role of innate immune cells, particularly macrophages. We observed a marked increase in CD11b+CD68+F480+ macrophages in both the E99K mouse model and HCM patients, along with enriched macrophage activation pathways in single-cell analyses, underscoring their involvement in HCM-associated fibrosis.

We employed a bone marrow chimera approach to investigate the role of monocyte/macrophage populations in HCM pathogenesis. Transplanting wild-type CD68-GFP+ bone marrow into irradiated E99K mice significantly improved systolic function, as indicated by enhanced ejection fraction (EF%) and peak systolic velocity (PSV), suggesting a reparative potential of wild-type bone marrow. Flow cytometry revealed an increase in CD68-GFP+ macrophages without elevated activation markers in the E99K mice, indicating that the transplanted wild-type bone marrow did not promote macrophage activation. In contrast, transplanting E99K bone marrow into wild-type mice led to a significant decline in heart function, along with increased monocyte/macrophage infiltration. FACS analysis revealed elevated activation markers such as MHCII and TLR2 exclusively in E99K-derived macrophages, suggesting a proinflammatory macrophage phenotype. These findings point to a potential mechanism by which infiltrating macrophages contribute to contractile dysfunction and adverse remodeling in HCM hearts.

We will examine BM-derived macrophages from E99K and WT mice to determine their role in remodeling. Targeting these macrophages could offer new therapeutic approaches to reduce myocardial fibrosis and improve HCM outcomes.

Poster number: 43

Spatially Resolved Insights Into Fistulating Crohn's Disease Pathogenesis

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Crohn's disease (CD) often presents with fistulae, abnormal tunnels connecting the intestine to the skin or other organs. Despite the profoundly adverse impact of fistulae on patient morbidity, the molecular basis of their formation remains unclear, largely due to the challenge of capturing intact fistula tracts and their inherent heterogeneity. Here, we construct an unbiased, subcellular-resolution spatial expression atlas of 62 intestinal fistulae spanning diverse anatomical locations. We describe fistula-associated epithelial, immune and stromal cell states, revealing abnormal zonation of growth factors and morphogens linked to establishment of tunnelling anatomy. We identified fistula-associated stromal (FAS) fibroblasts assembled in concentric layers, forming a proliferative, lumen-adjacent zone (LAZ) underlying surface neutrophil and macrophage-rich granulation tissue, followed by active lesion core FAS cells all encircled by quiescent, pro-fibrotic

outer zone (FOZ) fibroblasts. We mapped fistula tract ECM architecture and demonstrated FAS populations locate to different collagen structures, exhibiting functional properties spanning proliferation, migration and active ECM remodelling, to dense collagen deposition and fibrosis. We define niches supporting epithelialisation of fistula tunnels and a putative precursor FAS population detected at the base of ulcers in non-penetrating CD. This precursor population co-localises with inflammatory as opposed to pro-fibrotic SPP1+ macrophages and fails to induce developmental transcription factors, observed in FAS populations located in fistula tracts.

Poster number: 44

Trends in Cardiovascular Diseases and Risk Factors in the U.S. Adults From 1999 to 2023 by Age and Socioeconomic Status

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Background: Although trends in cardiovascular risk factors have been studied extensively, little is known about trends in cardiovascular diseases (CVDs). We present the first comprehensive evidence on CVDs and their risk factors simultaneously by age and socioeconomic status.

Methods: This study employed a serial cross-sectional analysis of adults aged 20–85 years in the U.S. participating in the National Health and Nutrition Examination Survey.

Results: A total of 47,743 adults were included in the analysis. Between 1999 and 2023, the prevalence of diabetes increased from 12.1% to 14.6%, body mass index (BMI) rose from 28.3 to 29.8, and hemoglobin A1c increased from 5.7 to 5.9. The prevalence of angina pectoris decreased from 4.4% to 2.3%, while smoking history significantly declined from 43.6% to 34.5%. Over the past decade, adults aged 45 to 64 years saw additional increases in the prevalence of coronary heart disease (CHD), congestive heart failure (CHF), and stroke. These trends were further exacerbated by socioeconomic disadvantage. Lower prevalence of CVDs and risk factors was associated

with women, Mexican Americans, individuals with a family income-to-poverty ratio > 3.0, those with a college education or higher, and those with private insurance.

Conclusions: Between 1999 and 2023 in the U.S., BMI, hemoglobin A1c levels and the prevalence of diabetes increased among adults. Over the past 10 years, this trend was largely driven by adults aged 45 to 64 years, with additional increases in the prevalence of CHD, CHF, and stroke. These trends were further exacerbated by socioeconomic disadvantage.

Poster number: 45

A Novel Deep Learning Model For Enhanced Segmentation of Internal Mammary Artery, Aorta And Their Perivascular Regions

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Aim: Previous study showed that a new radiotranscriptomic signature C19RS has prognostic value in clinical outcomes. Automatic segmentation of vascular structures, such as internal mammary artery (IMA), aorta and their perivascular regions, from contrast CT angiography (CCTA) would enable high throughout extraction of radiomic profile. We aim to develop a novel deep learning model for IMA and aorta that allows automated calculation of C19RS in large cohort analysis.

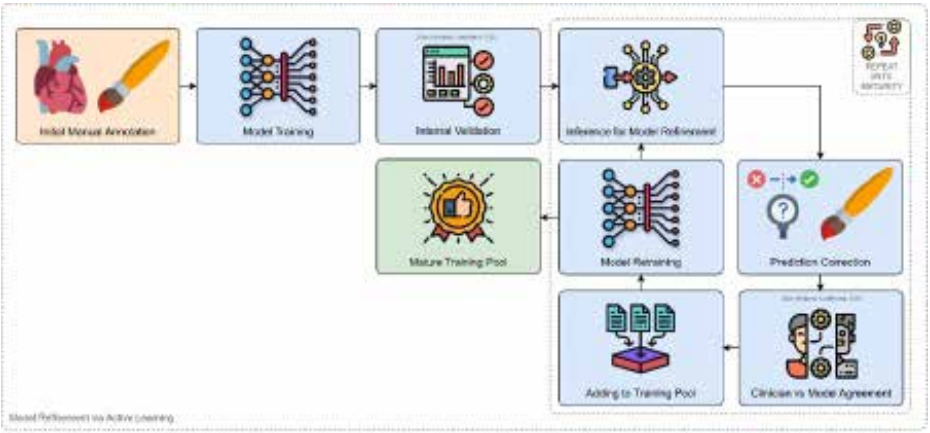
Methods: The model employs a unique architecture integrating CNN (squeeze-and-excitation block) and transformer (Swin block) to enhance segmentation by alternating the blocks and capturing discriminative features. It was trained on CCTA (n=227) dataset from the OxHVF study in the UK with standardized preprocessing steps (resampling, clipping, and intensity normalization). An iterative model refinement process was applied three times (n=140) to reach a mature model (see figure). An external validation cohort (n=751) from an internation site (United States) was used, with all segmentations undergoing manual expert review for quality assessment. Finally, a publicly available ASOCA dataset was externally validated (n=318).

Results: The model achieved a mean dice similarity score (DSC) of 0.7876±0.0176 for IMA/peri-IMA segmentation and 0.9207±0.0057 for the aorta/periaortic region segmentation (See figure). The model was refined reaching 0.947 for the IMA/peri-IMA segmentation. In the external cohort, 679 out of 751 cases (90.4%) were reported clinically acceptable for both regions, with the remaining excluded due to the absence of IMA/aorta in the narrow field of view CCTAs. In the ASOCA cohort, it showed consistent performance (0.961±0.039). These findings highlight the model's generalizability and scalability for large-scale clinical applications.

Conclusion: This study introduces a robust and clinically adaptable DL model for automated segmentation of vascular structures, including IMA, aorta, and their perivascular space. Its application in radiotranscriptomic biomarker

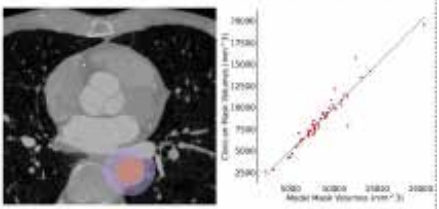
analysis offers a promising avenue for non-invasive patient outcome predictions, offering a valuable tool for cardiovascular research and clinical practice.

Figure 1. (Above) Iterative model refinement via active learning for reaching a segmentation model maturity before deploying in large-scale cohorts. (Below) The performance report and visualization of sample data.



Models	DSC↑	Precision↑	Recall↑
IMA/peri-IMA (1)	0.788±0.018	0.772±0.019	0.829±0.015
IMA/peri-IMA (2)	0.947±0.055	0.950±0.003	0.918±0.005
Aorta/periaortic	0.921±0.005	0.941±0.006	0.908±0.01
ASOCA cohort	0.961±0.039	0.961±0.007	0.938±0.009

Mean and standard deviation of DSC, recall, precision for 5-fold cross-validation. IMA/peri-IMA (1) and (2) show the performance before and after the model refinement via active learning.



Left figure shows a sample slice with IMA/aorta/peri-aorta. Right figure shows the volume comparison between model and clinical masks.

Poster number: 46

A Novel Variant in MYBPC3 Causes Hypertrophic Cardiomyopathy by Haploinsufficiency

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Rationale: Familial hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disease (CVD). Related mutations contributing to hypercontractility and poor relaxation in HCM are not completely understood. This study aimed to explore and verify a novel variant of cardiac myosin-binding protein C (cMyBP-C, encoded by MYBPC3) in an HCM family.

Methods: Clinical information and cardiac parameters were collected in the pedigree. Genomic DNA was extracted from peripheral blood and second-generation sequencing technology was used to investigate the proband and his family members. Subsequent sequence analysis was performed with DNAMAN software. Cardiac expression level of cMyBP-C was assessed using Western blot analysis.

Results: Typical interventricular septal thickening was detected in all four HCM patients without left ventricular outflow tract obstruction. The c.1042_1043insCGGCA mutation in MYBPC3 was verified in proband and family members. A mild phenotype associated with delayed onset but a high risk of sudden cardiac death was observed in the pedigree. In silico analysis of the mutation revealed that c.1042_1043insCGGCA led to a shift in the sequence of nucleotides, creating a premature stop codon at the new reading frame. Consistently, Western blot analysis showed significantly reduced expression of cMyBP-C in HCM hearts compared to the controls.

Conclusion: The novel c.1042_1043insCGGCA MYBPC3 mutation is a genetic basis for HCM due to c-MyBP-C haploinsufficiency.

Poster number: 47

Evaluating the genetic architecture shared between sleep-related traits, circulating glycemic biomarkers, and type 2 diabetes

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Background/rationale: Preclinical and observational studies suggest relationships of sleep traits with glycemic markers and type 2 diabetes (T2D); however, the genetic architecture linking these relationships remains unknown.

Methods & Results: We conduct colocalization screen genomic regions for evidence of shared causal variants and leverage both multi-omic data and functional approaches to resolve effector genes within the 18 unique genomic regions that colocalized. Among the sleep-glycemic trait pairs, insomnia risk and T2D were linked by a missense variant in the PATJ locus, rs12140153, which was robust to replication in additional GWAS datasets. Local genetic correlations and cis-instrument Mendelian randomization for each of the 18 loci informed these direction Zebrafish larvae with CRISPR-mediated knockout of PATJ demonstrated dysregulated sleep episodes and metabolite levels relative to controls, supporting a causal role of PATJ in sleep and metabolism. 5 other effector genes, FTO, RBM6, CACNA1D, and IP6K1-APEK were also prioritized by multi-trait colocalization that integrated expression quantitative trait loci from liver, muscle, adipose, pancreatic islets, and brain tissues. Extensive phenotyping of the effector genes in biobanks and circadian rhythm databases supported their involvement of the effector genes in sleep-related

behaviors and cardiometabolic traits, e.g., PATJ variants influenced adiposity in the Oxford Biobank.

Conclusions: These results provide biological insight into the relationships of sleep traits and T2D that may have important implications for therapeutic strategies aiming to reduce hyperglycemia and T2D in the context of sleep and circadian rhythms.

Poster number: 48

The glucagon-like peptide-1 receptor antagonist, Exendin (9-39)NH2, improves the impaired glucagon response to hypoglycaemia in type 1 diabetes

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Rationale: In type 1 diabetes (T1D), counterregulatory islet alpha-cell glucagon secretion becomes insufficient, contributing to potentially fatal hypoglycaemia. Normally, glucagon-like peptide-1 (GLP-1) is not predominantly produced by alpha-cells due to the selective expression of prohormone convertase-2, which processes proglucagon to glucagon. Recent observations suggest that activity levels of prohormone convertase-1/3, an enzyme that processes proglucagon to GLP-1, are increased in T1D alpha-cells. We investigated the contribution of intra-islet GLP-1 signalling on glucagon secretion during hypoglycaemia in islets from the autoimmune non-obese diabetic (NOD) mouse and human T1D donors.

Methodology: Islet hormone secretion was assessed in adult diabetic (blood glucose ≥ 25 mmol/l) and normoglycaemic (blood glucose 5-8 mmol/l) pre-diabetic control NOD/ShiLtJ mice by in situ pancreas perfusion and from human T1D and non-diabetic islets by in vitro static secretion.
Results: While hypoglycaemia normally increased glucagon secretion from the perfused NOD control pancreas, this response was abolished from the diabetic pancreas (10 mmol/l vs 1 mmol/l glucose: control, 9.17±1.36 vs 21.5±4.32 pg/min, n = 7; diabetic, 3.99±1.78 vs 6.03±0.22 pg/min, n = 5). The GLP-1R antagonist, exendin (9-39) amide, significantly improved hypoglycaemia-induced glucagon secretion from the diabetic NOD pancreas by 3.5-fold (*p < 0.05, n = 5 mice) and from human T1D islets by 1.9-fold (*p < 0.05, n = 2 T1D donors).

Conclusion: An increase in intra-islet GLP-1 signalling plays a significant role in the defective glucagon response to hypoglycaemia seen in T1D and provides a rationale for GLP-1R antagonists as a promising adjunct therapy to insulin for reducing the risk of hypoglycaemia in individuals with T1D.

Poster number: 49

Mechanoregulation of lymphatic valve development and lymphoedema

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Primary Lymphoedema is a lymphovascular disease caused by an underlying abnormality of the lymphatic system, which is inherited, and clinically and genetically heterogenous. Crucially, of all patients tested for Primary Lymphoedema, only 41% are given a molecular diagnosis. A common feature of these patients is insufficient or inadequate lymphatic valve (LV) formation or function. LVs are initially formed by lymphatic endothelial cells (LECs) which express lymphatic valve-forming genes. The stimulus responsible for activation of the LV programme and formation of LVs is fluid shear stress due to lymph fluid, however, the molecular mechanisms by which shear stress induces LV formation are a mystery. We now show that PLXND1 functions as a mechanosensor in LECs and is required for LV formation. We also identify variants in PLXND1 that are associated with lymphoedema.

1. Martin-Almedina, et al. Physiol Rev 101, 1809-1871 (2021)

2. Yang, Y. et al. Cell Rep 28, 2397-2412 e2394 (2019)

Poster number: 50

Shifting Target: Baseline brain health influences the volume of oedema required to cause midline shift after ischaemic stroke

Authors: James Garrard (Doctor of Medicine Student)1, Davide Carone1, James Kennedy1

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Rationale: Midline shift (MLS) is widely used to assess cerebral oedema following ischaemic stroke in clinical practice and trials. However, the influence of baseline brain health on the volume of oedema required to induce MLS is unclear. This study aimed to quantify this relationship and assess the role of baseline brain health.

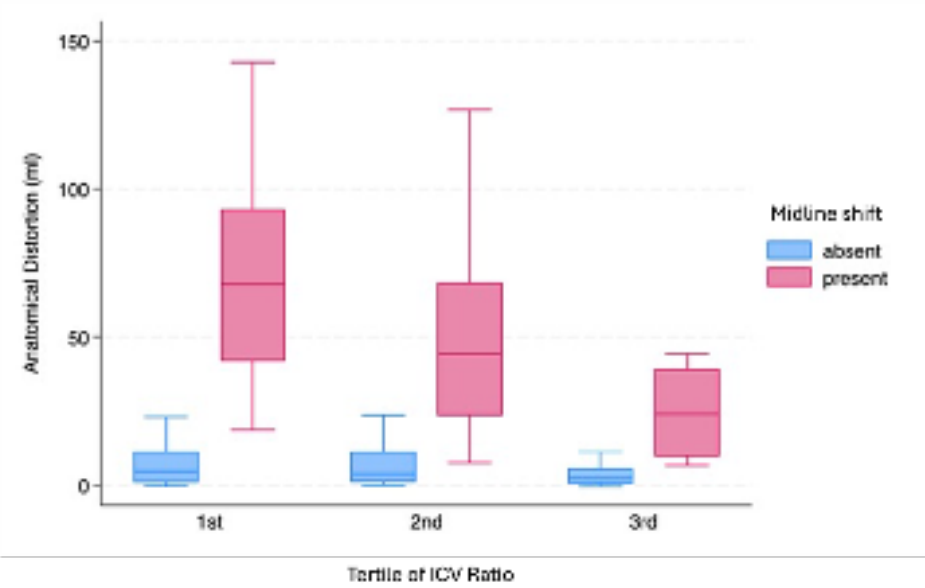
Methods: Ischaemic stroke patients who received thrombolysis and/or mechanical thrombectomy with available baseline and follow-up (24-36 hours) imaging were included. Baseline brain health was measured using the brain-to-intracranial volume ratio (ICVR). A neuroradiologist assessed follow-up scans for midline shift and infarct segmentation. Anatomical distortion (AD), a volumetric measure of oedema, was calculated as the difference between infarct mask volumes following linear and non-linear image co-registration. The relationship between AD and MLS was analysed, adjusting for ICVR.

Results: Among 348 patients (mean age: 70 (SD=12.9); 160(46%) females; 72(20.8%) with diabetes, lower ICVR was significantly associated with increasing age ($p<0.001$) and diabetes ($p=0.002$). The average ICVR was 0.81±0.06 and split into tertiles: <=0.77; 0.77-0.84; and >=0.84.

AD was significantly higher in patients with MLS in all tertiles ($p=0.001$). The volume of AD in patients with MLS increased as ICVR decreased (Tertile 1=67.8[41.7-93.1], Tertile 2=44.1[23.3-68.4], Tertile 3=23.8[0.5-39.3] ($p=0.008$) (Figure 1).

Conclusions: Baseline brain health influences the oedema threshold for MLS and should be considered in clinical trials evaluating oedema to reduce false-negative outcomes. AD provides a volumetric measure linked to MLS, warranting further investigation into its clinical relevance.

Figure 1. Boxplot of Anatomical Distortion for each tertile of ICV Ratio, split by the presence or absence of midline shift



Poster number: 51

Evaluating the pathogenic significance of unique chromosomal variants in craniosynostosis using patient-derived induced pluripotent stem cell lines and mouse modelling

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†These authors co-supervised the work

Purpose: Craniosynostosis (premature fusion of cranial sutures) is associated in ~2.5% of patients with chromosomal rearrangements such as copy number variants (CNVs), but demonstrating a causative role for specific CNVs in disease can be challenging, yet essential for correct genetic counselling. We investigated two families in which patients with craniosynostosis had SV/CNV potentially dysregulating a fibroblast growth factor (FGF)-encoding gene; a mother and daughter segregating a 730 kb duplication on 4q21.21 including FGF5; and a sporadic patient with a 568 kb interspersed duplication on 13q12.11, located 841 kb from FGF9.

Methods: We generated induced pluripotent stem cell lines (iPSCs) from affected individuals and differentiated them to neural crest and osteogenic identities. The effect on gene expression and chromatin accessibility was analysed using RNA and ATAC sequencing. For the 4q21.21 duplication we generated a mouse bearing an equivalent rearrangement using CRISPR-Cas9 targeting.

Results: After differentiating 4q21.21 duplication iPSCs to both neural crest and osteogenic identities, FGF5 exhibited the highest-fold upregulation of expression of any of 21 genes present in a 2 Mb window flanking the CNV. Mice with the equivalent duplication had multisuture craniosynostosis and

mutant embryonic coronal sutures exhibited increased Fgf5 expression. For 13q12.11 SV iPSCs, FGF9 expression was unremarkable compared with nearby genes, and a de novo pathogenic variant in FOXP2 provided a plausible alternative explanation of the patient's phenotype.

Conclusion: Both positive and negative findings from iPSC-derived data can provide valuable functional evidence when evaluating a causal link between unique SV/CNV and craniosynostosis.

Poster number: 52

Developing Genetic Screens in Infant Acute Lymphoblastic Leukaemia

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MLL-rearrangements constitute an aggressive and treatment refractory form of acute lymphoblastic leukaemia (ALL) in infants1. Approximately half of these cases are caused by the MLL-AF4 translocation which aberrantly upregulates oncogenes to maintain an infant-specific gene expression profile. This program is regulated directly via DOT1L and indirectly via Menin2,3. Although, Menin inhibitors have recently entered clinical use4, more work is needed to understand the pathobiology of MLL-AF4 driven ALL.

CRISPR screening technology has been instrumental in identifying new targets for therapeutic intervention. In this study we aim to identify novel genetic sensitivities of MLL-AF4-driven infant ALL using targeted CRISPR screens. We employ the use of a novel primary human MLL-AF4 ALL model that provides a biologically relevant context and recapitulates the pathogenic infant-specific gene expression profile characterising infant ALL2. Here we outline the results of a small-scale CRISPR screen targeting DNA damage response genes in the human MLL-AF4 SEM cell line. To identify putative disease-specific genetic dependencies we compare the cell line data to screen data obtained from healthy haematopoietic stem and progenitor cells. In the future we aim to validate the putative hits from these initial screens and perform additional targeted CRISPR screens in the primary MLL-AF4 ALL model

Citations:

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Rice S, Jackson T, Crump NT, Fordham N, Elliott N, O’Byrne S, et al. A human fetal liver-derived infant MLL-AF4 acute lymphoblastic leukemia model reveals a distinct fetal gene expression program. Nat Commun. 2021.12(1):6905.

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Poster number: 53

High thrombopoietin restricts preleukemic proliferation to the foetal liver in Down's syndrome

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Paediatric cancers differ from adult cancers in their genetics and biology. Understanding these differences helps identify key oncogenic factors during development. Prime examples are the genetically linked myeloid pre-leukemic and leukemic disorders in newborns and children with Trisomy 21 (T21) or Down syndrome (DS). Children with DS have a 400-fold higher risk of developing myeloid leukaemia (ML-DS) early in life. ML-DS is preceded by a foetal pre-leukemic phase, known as transient abnormal myelopoiesis (TAM), when T21 cells acquire GATA1 mutations. Notably, T21 and GATA1 mutations drive proliferation only during foetal development. As hemopoiesis shifts from the foetal liver to the newborn bone marrow, TAM usually regresses. The reason TAM cells depend on the foetal liver but fail to persist in the bone marrow remains unknown.

To investigate this, we performed scRNAseq on TAM samples. Compared to controls, TAM cells expanded at the GATA1-expressing progenitor stage and showed JAK/STAT pathway upregulation. We hypothesized that aberrant cytokine receptor activation drives TAM cell proliferation. Differential gene expression pointed to CSF2RB, a cytokine receptor that directly activates JAK/STAT signalling. CSF2RB was overexpressed in TAM cells, marking 80% of CD34+ cells versus 5% in controls.

CSF2RB typically partners with IL3, IL5, or GM-CSF receptors, but these were absent in TAM cells. Instead, we identified the thrombopoietin (TPO) receptor (TPOR) as a novel CSF2RB partner based on: (i) co-expression of TPOR and CSF2RB in TAM cells, (ii) direct TPOR-CSF2RB interaction via BRET and co-IP assays, (iii) TPOR-CSF2RB internalization upon TPO treatment, (iv) CSF2RB enhanced TPO signalling in Ba/F3 and TF-1 models, specifically at high TPO and (v) a mutation in CSFR2RB (A455D) identified in ML-DS requires interaction with TPOR to induce autonomous growth of cytokine-dependent

hematopoietic cells. Additionally, TAM cells proliferated more with increasing TPO levels, and CSF2RB overexpression in TAM cells enhanced STAT3/5 phosphorylation specifically at high TPO.

We propose that high levels of foetal liver-derived TPO sustain TAM cell proliferation via the TPOR-CSF2RB interaction. Loss of this proliferation signal as cells migrate to the low TPO bone marrow environment creates a selective pressure for acquisition of secondary mutations that permit TAM cells to proliferate independently of the high TPO environment of the foetal liver. These mutations transform the TAM clone to an ML-DS clone.

Poster number: 54

Hypercholesterolemia-induced trained immunity in hematopoietic stem cells mediates sustained macrophage inflammation and adipose tissue metabolic dysfunction refractory to cholesterol lowering

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In patients with atherosclerotic cardiovascular disease (ASCVD) lowering LDL cholesterol (LDL-C) reduces the risk of adverse cardiovascular events. However, these patients remain at continued risk of adverse cardiovascular events despite achieving optimal LDL-C lowering. We hypothesised that this 'residual risk' is mediated by programmed effects on immune cell metabolism and polarisation, is not resolved by cholesterol lowering and has adverse systemic effects. We found that exposure to high cholesterol (HC) in vivo induced long-term metabolic changes in macrophages differentiated ex vivo from bone marrow, leading to a pro-inflammatory profile; these changes persisted despite cholesterol lowering. Chromatin accessibility was altered in hematopoietic stem cells (HSC) from HC mice, that persisted after cholesterol lowering and was evident in bone marrow monocytes and tissue resident macrophages. HC provoked RUNX1 dependent regulation of stearoyl-CoA desaturase (SCD) reduced mono-unsaturated fatty acid (MUFA) availability for OxPhos in monocytes and macrophages, whereas supplementing with MUFA restored OxPhos capacity and reduced macrophage pro-inflammatory phenotype. Furthermore, HC-exposed HSC conferred adverse systemic metabolic effects in normocholesterolemic mice, with increased adipose tissue mass and glycaemic dysfunction, via adipose tissue repopulation with HC-exposed macrophages that increased adipose tissue inflammation. These findings indicate that elevated LDL-C results in long-lasting immuno-epigenetic memory in HSC which is refractory to lipid lowering, and provide strong evidence that exposure to high cholesterol can have prolonged consequences that require new therapeutic approaches beyond LDL-C lowering.

Poster number: 55

Novel AI technology to quantify coronary inflammation and cardiovascular risk with routine Coronary Computed Tomography Angiography: a cost-effectiveness analysis

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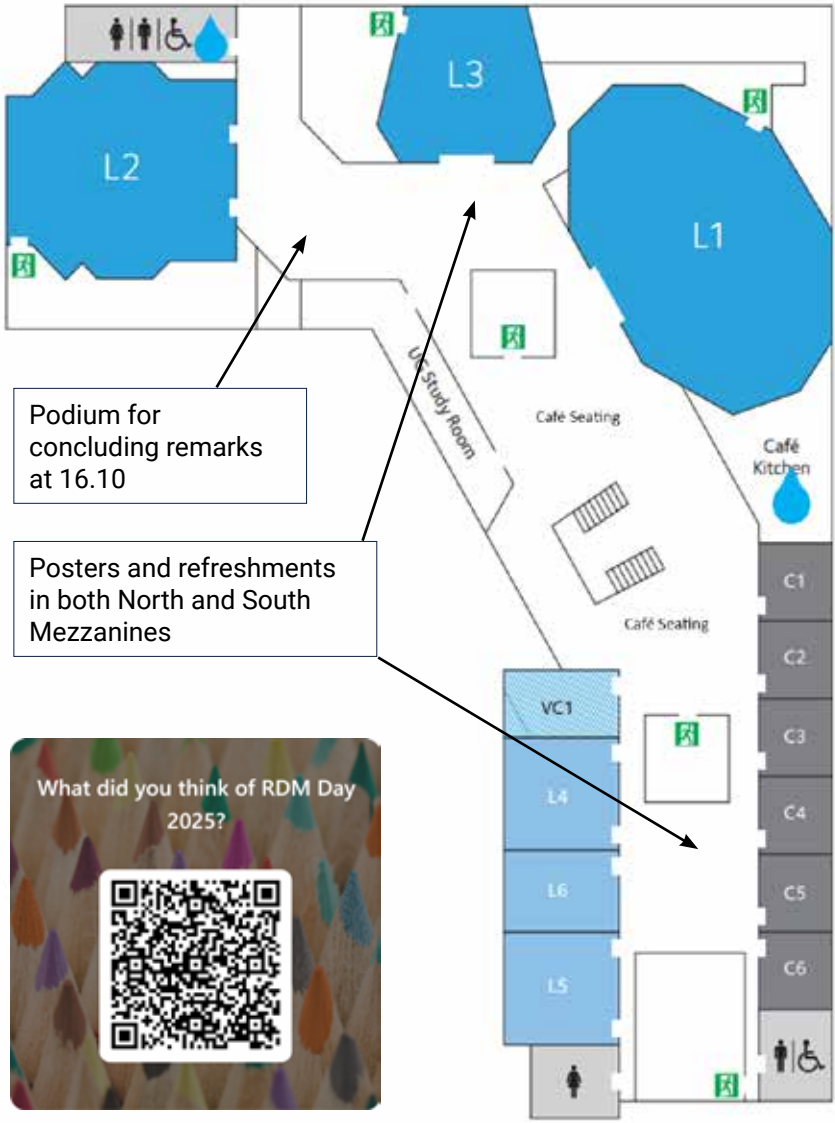
Background: Coronary Computed Tomography Angiography (CCTA) is a first-line investigation for chest pain in patients with suspected obstructive coronary artery disease (CAD). However, many acute cardiac events occur in the absence of obstructive CAD. We assessed the lifetime cost-effectiveness of integrating a novel AI-enhanced image analysis algorithm (AI-Risk) that stratifies the risk of cardiac events by quantifying coronary inflammation from routine CCTA, combined with the plaque burden and clinical risk factors.

Methods: A Markov model was developed from 3,393 consecutive patients who underwent routine CCTA for suspected obstructive CAD and followed for a median(IQR) of 7.7(6.4-9.1) years. The observed clinical events were modelled over a lifetime horizon to evaluate the clinical impact of AI-Risk guided treatment pathway compared with standard care.(Figure) A prospective real-world evaluation survey on 744 consecutive patients undergoing CCTA for chest pain investigation was performed to capture

changes in treatment decisions after AI-Risk assessment. A further prospective study of 1,214 consecutive patients with extensive guideline cardiovascular risk profiling was performed to evaluate how AI-Risk classification can change patient management when applied on top of full compliance to UK NICE guideline.

Results: AI-Risk guided management led to fewer cardiovascular events compared with standard care (relative reductions of 11%, 4%, 4%, and 12% for myocardial infarction, stroke, heart failure and cardiac death respectively). AI-Risk led to treatment initiation or intensification in 39% patients beyond current clinical guideline recommendations. Implementing AI-Risk classification in routine interpretation of CCTA is highly likely to be cost-effective [Incremental cost-effectiveness ratio £3244 for the baseline model, and £3103 when full guideline compliance for the baseline model is assumed.

Conclusion: The addition of AI-Risk assessment in routine CCTA interpretation is cost-effective compared to standard care by refining risk-guided medical management.


L2
L3
L1
UG Study Room
Café Seating
Café Kitchen
Café Seating
C1
C2
C3
C4
C5
C6
VC1
L4
L6
L5
Podium for concluding remarks at 16.10
Posters and refreshments in both North and South Mezzanines
What did you think of RDM Day 2025?