Oral presentations

Single-cell multi-omics in pancreatic cancer reveals differential immune evasion mechanisms between prognostic groups

Shivan Sivakumar

Department of Oncology, University of Oxford, UK

Pancreatic cancer has the worst survival of any human cancer with a 5 year survival of less than 10% with minimal treatment options. We have previously made the observation that patients with a T cell infiltrate in their primary tumour have a better prognosis then those that do not. To understand the nature of the immune infiltrate, we have in 12 patients performed 10X sequencing on CD45 cells from the tumour as well as single cell TCR-seq, BCR-seq and cite-seq and have performed the same experiment on the matching PBMCs. We have generated a dataset of approximately 185000 cells with half coming from the tumour. This unravels a complex immune infiltrate with the predominant immune cell types consisting of T cells. Our data shows that within the tumour infiltrate, patients either have high or low myeloid infiltration. We see a highly active Treg population that has multiple checkpoints activated. Using TCR data, we saw the biggest clonal expansions in CD8 EM, CD8 senescent cells and Tregs. Circulating TIL CD4 T cells are dominated by activated Tregs, Tfh, and Th2 and circulating TIL CD8 T cells are dominated by CD8 EM T cells. Our work identifies multiple novel therapeutic targets that should form the basis for rational design of a new generation of clinical trials in pancreatic ductal adenocarcinoma.

Use of live tumour slices for the optimisation of CAR-T cell therapy in PDAC

Evans A, Hughes D, O'Neill E

Department of Oncology, University of Oxford, UK

Chimeric antigen receptor (CAR) T cell therapy has markedly improved the prognosis of haematological malignancies. The immune suppressive environment and dense stromal barrier in cancers such have pancreatic ductal adenocarcinoma (PDAC) have prevented translation of this success into solid tumour settings. We currently lack representative pre-clinical models preventing the analysis of the most important factors hindering CAR-T cell efficacy.

Organotypic tumour slices allow interrogation of therapeutics in a model faithful to the native tumour. The system allows preservation of cellular heterogeneity, local cytokine landscape and stromal structure. We are using dynamic perfusion culture of these tumour slices to analyse the efficacy of a novel CAR-T cell construct targeting the 5T4 oncofoetal antigen in PDAC.

We have shown improvement in tumour slice viability in dynamic over static culture, with viability maintained over a period of 7-12 days. Interrogation of 5T4 expression across patient tumours has shown widespread expression across tumours and absence in healthy pancreatic tissue, supporting both the safety and efficacy of the target. We have additionally developed IF protocols to examine the location and tissue penetrance of exogenous T cells applied to tumour slices. Further work will involve generation of 5T4 CAR-T cells from matched patient blood and application to tumour slices. Live imaging methodology will be developed alongside cytokine array analysis to determine major barriers preventing CAR-T cell efficacy.

Segmented filamentous bacteria-induced epithelial MHCII regulates cognate CD4+ intraepithelial lymphocytes and epithelial turnover

Tomas Brabec[1], Martin Schwarzer[2], Katarína Kováčová[1], Martina Dobešová[1, 3], Dagmar Schierova[4], Jiří Březina[3], Iva Pacáková[1], Dagmar Šrůtková[2], Osher Ben-Nun[5], Yael Goldfarb[5], Iva Šplichalová[3], Michal Kolář[6], Jakub Abramson[5], Dominik Filipp[3] and Jan Dobeš[1]

[1]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic; [2]Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Nový Hrádek, Czech Republic; [3]Laboratory of Immunobiology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic; [4]Laboratory of Anaerobic Microbiology, Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Prague, Czech Republic; [5]Department of Immunology, Weizmann Institute of Science, Rehovot, Israel; [6]Laboratory of Genomics and Bioinformatics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Intestinal epithelial cells have the capacity to upregulate MHCII molecules in response to certain epithelial-adhesive microbes, such as segmented filamentous bacteria (SFB). However, the mechanism regulating MHCII expression as well as the impact of epithelial MHCII-mediated antigen presentation on T-cell responses targeting those microbes remains elusive. Here we identify the cellular network that regulates MHCII expression on the intestinal epithelium in response to SFB. Since MHCII on the intestinal epithelium is dispensable for SFB-induced Th17 response, we explored other CD4+ T-cell-based responses induced by SFB. We have found that SFB drive the conversion of cognate CD4+ T-cells to Granzyme+ CD8α+ intraepithelial lymphocytes. These cells accumulate in small intestinal intraepithelial space in response to SFB. Yet, their accumulation is abrogated by the ablation of MHCII on the intestinal epithelium. Finally, we show that this mechanism is indispensable for the SFB-driven increase in the turnover of epithelial cells in the ileum. This study identifies a previously uncharacterized immune response to SFB, which is dependent on the epithelial antigen presentation.

Immunomodulatory Adipose Tissue Perineurial Cells Protect Against Obesity

Emma Haberman[1], Bernardo Arús[2], Gitalee Sarker[1], Eliška Freibergerova[1], Iara Fernández-González[3], Miguel López[3], Ana Domingos1[*]

[1]University of Oxford, UK; [2]Instituto Gulbenkian de Ciência, Portugal; [3]Universidade de Santiago de Compostela, Spain

Obesity is a culmination of long-term energy imbalance, resulting in the storage of excess fats as triglycerides in adipose tissues (ATs). Sympathetically innervated ATs are key sites of both energy storage and hormone production. Leptin is one such hormone produced by adipocytes, released in proportion to AT mass. Leptin acts in the hypothalamus to diminish hunger and increase sympathetic drive onto ATs. This increase in sympathetic-derived norepinephrine stimulates adipocyte lipolysis and thermogenesis, providing a neuroendocrine negative feedback loop which broadly controls energy storage and energy expenditure. Obesity is closely associated with chronic, low-grade AT inflammation, mirrored by an increase in pro-inflammatory and a concomitant decrease in anti-inflammatory AT immune cells. Here, we reveal a novel population of Leptin receptor+ perineurial cells ensheathing AT sympathetic neurons, which produce key anti-inflammatory cytokines, including IL33. We show that mice with a perineurial cell specific loss of IL33 gain more weight when fed high-fat diet despite comparable food intake - indicative of metabolic dysfunction. In the pre-obese state, a loss of perineurial cell derived IL33 reduces the frequency of anti-inflammatory regulatory immune cells, including regulatory T cells (Tregs) specifically within the brown adipose tissue (BAT). Along with this shift in BAT-populating immune cells, we show that these mice have impaired BAT thermogenesis, predisposing to obesity with both age and metabolic challenge. Together, this firmly implicates perineurial cells in the regulation of adipose tissue homeostasis.

Etiology of Type 1 Diabetes: Tolerance to insulin is hardwired by mimicry into microbial metabolism of carbohydrates

Arcadio Rubio García[1], Athina Paterou[1], Rebecca D. Powell Doherty[2], Laurie G. Landry[4], Mercede Lee[1], Amanda M. Anderson[4], Hubert Slawinski[3], Ricardo C. Ferreira[1], Dominik Trzupek[1], Holly Roach[1], Agnieszka Szypowska[7], Linda S. Wicker[1], Luc Teyton[8], Nicola Ternette[2], Maki Nakayama [4-6], John A. Todd (1), & Marcin L. Pekalski ([1,9])

[1]JDRF/Wellcome Diabetes and Inflammation Laboratory, Wellcome Centre for Human Genetics, Nuffield Department of Medicine, NIHR Biomedical Research Centre, University of Oxford, UK
[2]The Jenner Institute, Centre for Immuno-Oncology, Old Road Campus Research Building, University of Oxford, UK
[3]Wellcome Centre for Human Genetics, Nuffield Department of Medicine, NIHR Biomedical Research Centre, University of Oxford, Oxford, UK
[4]Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, Colorado, USA
[5]Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado, USA
[6]Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, Colorado, USA
[7]Department of Pediatrics, Medical University of Warsaw, Warsaw, Poland
[8]Department of Immunology and Microbiology, Scripps Research Institute, La Jolla, California, USA
[9]Department of Paediatrics, University of Oxford, UK

The gut microbiome of individuals from industrialised societies is characterised by a reduced biodiversity, making it a fragile ecosystem prone to imbalances that may translate into abrupt changes in its functional composition and metabolic activities. These maladaptive states, broadly described as dysbiosis, have been associated with the increasing incidence of many common disorders, most notably autoimmune ones, including type 1 diabetes (T1D). Here, we identify a host-microbiome interaction fundamental to the aetiology of T1D, classically considered an HLA class II-regulated, T-cell-mediated destruction of pancreatic β cells, owing to a loss of immune tolerance to insulin and other islet antigens. We describe a large set of gut commensal proteins, enriched with members of the transketolase enzyme superfamily (TKT), that contain peptide sequences whose similarity to the primary target of T1D autoimmunity, insulin B:9-25 peptide, is statistically significant. We demonstrate that islet infiltrates from early stage T1D contain T cells cross-reactive to bacterial TKT and insulin peptides. Furthermore, we link T1D risk due to HLA-DQ, which is largely determined by the charge of the amino acid at position 57 of the DQ β-chain, to specific T cell repertoire biases that increase the proportion of clones reactive to insulin that escape thymic selection. TKT is highly upregulated during infant weaning, a period preceding the peak incidence of insulin autoantibodies. Restoration of early life gut ecology is being trialled for T1D prevention based on these findings. We anticipate equivalent discoveries in other diseases associated with dysbiosis and HLA polymorphisms, such as Parkinson's and Alzheimer's.

Lightning talks

P.01 **Single nuclei RNA-seq differentiates between mouse models of lupus nephritis and highlights pathways for therapeutic intervention**

Ms Aneesha Bhandari[1], Dr Rose Hodgson[1], Dr Mukta Deobagkar[2], Ms Tanya Cheetham[2], Professor Richard Cornall[1], Dr Katherine Bull[2]

[1]Nuffield Department of Medicine, University of Oxford, UK; [2]Wellcome Centre for Human Genetics, University of Oxford, UK

Introduction
Lupus Nephritis (LN) is characterised by renal immune-complex deposition, but how these deposits trigger inflammatory mediators, and the signalling between resident and recruited cells remains unclear. Mouse lupus models share many of the clinical features of human disease including renal involvement. In this study, using single nuclei RNA sequencing (snRNA-seq), we compare two different murine models of early LN, to identify disease drivers, potential therapeutic targets, and to establish an approach that can be applied to human tissue in the future.

Methods
Kidneys were harvested from autoimmune (MRL/lpr) mice aged 16 weeks, or Balb/c mice treated with 8 weeks topical TLR7 agonist (IMQ) with respective controls. Kidney snRNA-seq libraries were prepared, sequenced, and analysed.

Results
Transcriptomic data identified significantly enriched intrarenal T, B, and myeloid cells in diseased kidney. Shared immune renal phenotypes included increased resident macrophages expressing genes implicated in phagocytosis and efferocytosis (Axl,Mertk) and antigen presentation (H2-Eb1) and patrolling non-classical monocytes, expressing proinflammatory transcription factor Nfam. MRL/lpr mice had the largest immune infiltrate, including CD8 and NK cells expressing markers of cytotoxicity and exhaustion (Gzma, Klrg1). In contrast in IMQ kidney there were significant increases in innate populations, and a unique Fcrl5+ monocyte signature.

Discussion
Integrated kidney snRNA-seq in two LN models delineates a shared immune signature, with interactions with stromal, tubular, and glomerular cells important in early disease perpetuation and damage. Our comprehensive kidney single nuclei approach is generalisable and highlights candidate genes for rescue experiments. A similar integrated approach can be applied to human pathology.

P.02 **Delayed booster dosing enhances circulating and bone marrow-resident human B cell responses to blood-stage malaria vaccine antigens**

JR Barrett[1], SE Silk[1]) CG Mkindi[2], KM Kwiatkowska[1], MM Hou[1], AM Lias[1], WF Kalinga[2], IM Mtaka[2], H Davies[1], L King[1], N Edwards[1], S Rwezaula[3], CE Chitnis[4], AO Olotu[2], AM Minassian[1], SJ Draper[1], CM Nielsen[1]

[1]University of Oxford, Oxford, UK; [2]Ifakara Health Institute, Bagamoyo, Tanzania; [3]Muhimbili National Hospital, Dar es Salaam, Tanzania; [4]Unité de Biologie de Plasmodium et Vaccins, Institut Pasteur, Université Paris Cité, Paris, France

We have previously reported primary endpoints of a clinical trial testing two vaccine platforms for delivery of Plasmodium vivax malaria DBPRII: viral vectors (ChAd63, MVA) and protein/adjuvant (50µg Matrix-MTM). Delayed boosting was necessitated due to trial halts during the pandemic and provides an opportunity to investigate the impact of dosing regimens. Here, using flow cytometry – including agnostic definition of populations with clustering tool CITRUS – we report enhanced induction of DBPRII-specific plasma cell and memory B cell responses in protein/adjuvant versus viral vector vaccinees. Within protein/adjuvant groups, delayed boosting further improved B cell immunogenicity as compared to the monthly regimen. Consistent with this, delayed boosting also drove more durable anti-DBPRII serum IgG. In an independent vaccine clinical trial with Plasmodium

falciparum malaria RH5 protein/adjuvant (50µg Matrix-MTM) vaccine candidate, we similarly observed enhanced circulating B cell responses in vaccinees receiving a delayed final booster. Notably, a higher frequency of vaccine-specific (putatively long-lived) plasma cells were detected in the bone marrow of these delayed boosting vaccinees by ELISPOT and correlated strongly with serum IgG.

Finally, following controlled human malaria infection with P vivax parasites in the DBPRII trial, in vivo growth inhibition was observed to correlate with DBPRII-specific B cell and serum IgG responses. In contrast, the T cell response was impacted by vaccine platform but not dosing regimen, and did not correlate with IVGI. Taken together, our DBP and RH5 data suggest an opportunity for dosing regimen optimisation in the context of rationale vaccine development against pathogens where protection is antibody-mediated.

P.03 **Adipocyte autophagy in the control of obesity-induced inflammation**

Klara Piletic[1], Felix C. Richter[1], Lada Koneva[1], Stephen Sansom[1], Fiona Powrie[1], Ghada Alsaleh[1], & Katja Simon[1, 2]

[1]Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK; [2]Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Autophagy is a cellular recycling process that is fundamental for cellular differentiation, homeostasis, and function. In recent years, the importance of autophagy in mediating communication between cells and organs through the provision of nutrients and signalling molecules has become increasingly recognized. However, how autophagy engages in the crosstalk between inflammatory cells and tissues to regulate chronic inflammation remains poorly understood. Obesity-related chronic inflammation in human and murine models correlates with enhanced levels of autophagy in adipose tissue. We found that adipocyte specific depletion of autophagy in mice at the onset of obesity results in a metabolically healthier obesity, characterized by a more favourable body fat distribution, improved insulin sensitivity, and reduced ectopic fat deposition. These effects are likely orchestrated by adipose tissue fibrosis, which is highly exacerbated in the absence of adipocyte autophagy. Surprisingly, at the level of intercellular communication, we observe that increased adipocyte autophagy supports the inflammation of visceral adipose tissues by controlling macrophage and T cell number, heterogeneity, and function. This intercellular communication is plausibly controlled through secretory autophagy, as in vitro stimulation of macrophages with conditioned medium from adipocytes without autophagy recapitulates in vivo observations. At the moment, we predict that the inflammation is induced via an anti-inflammatory effector molecule which autophagy controls. These observations position adipocyte autophagy as a mediator of metabolic syndrome and adipose tissue inflammation in obesity. While the molecular underpinnings of these interactions remain to be understood, they may uncover new opportunities for the treatment of visceral obesity-related complications.

P.04 **Computationally profiling peptide:MHC recognition by T-cell receptors and T-cell receptor-mimetic antibodies**

Matthew I. J. Raybould[1], Daniel A. Nissley[1], Sandeep Kumar[2], Charlotte M. Deane[1]

[1]Oxford Protein Informatics Group, Department of Statistics, University of Oxford, UK
[2]Biotherapeutics Discovery, Boehringer Ingelheim, Ridgefield, Connecticut, USA

Peptides presented by the Major Histocompatibility Complex (pMHCs) are set to become a major class of drug/diagnostic target in oncology; when neoantigen peptides are displayed they enable pinpoint distinction between cancerous and healthy cells.

Membrane-bound T-cell receptors (TCRs) are selected by nature to recognise pMHC targets with high peptide sensitivity via a canonical diagonal binding mode that scans many peptide residues. However, exploiting TCRs as ‘off-the-shelf’ soluble cancer diagnostics/therapeutics requires substantial engineering and affinity enhancement, and their drug development pipelines remain

nascent. An alternative strategy to target pMHCs is to use antibodies (known as TCR-mimetics, TCRms). Antibodies routinely bind their antigens with the affinity required of soluble drugs and benefit from decades of research into their preclinical development. However, antibodies have not evolved for the explicit purpose of recognising MHC-presented molecules.

By analysing the latest available structural data, we demonstrate that idiotypic differences in the structures of antibodies and TCRs directly influence the pMHC recognition event, leading to profiles that suggest, on average, an increased risk of off-target binding for TCRms. Nonetheless, many pMHC recognition features of a subset of TCRms, including the first two TCRms to enter clinical trials, are remarkably 'TCR-like'. We also identify a conserved interaction to a common MHC allele associated with guiding TCRms towards more TCR-like recognition.

Overall, our study provides the first set of benchmarks for the rational computational de-risking of affinity-enhanced TCR and TCR-mimetic modalities, and a novel strategy for screening library design that should more frequently yield TCRm candidates with enhanced TCR-likeness.

P.05 **Single cell profiling of transplant biopsies following adoptive regulatory T cell therapy**

Oliver McCallion, Matthew Brook, Joanna Hester, Fadi Issa

Nuffield Department of Surgical Sciences, Medical Sciences Division, University of Oxford, UK

BACKGROUND: Adoptive cell therapy (ACT) with autologous expanded regulatory T cells (Tregs) is a promising strategy to enable immunosuppression minimisation in organ transplant recipients. This approach is currently being assessed in a phase II RCT in Oxford. In these patients, there is a need to assess the migration patterns of transferred Tregs, and their impact on tissue alloresponses within the transplant.

AIM: To compare the clonality of adoptively transferred and transplant-infiltrating Tregs and assess their impact on the cell states of tissue-resident leukocytes.

METHODS: Cryopreserved 38-week core biopsies from patients receiving standard-of-care immunosuppression (n=2) or Treg ACT (n=1) were enzymatically dissociated and CD45+ lymphocytes isolated by flow sorting. Paired PBMC and Treg ACT single cell suspensions were generated from which gene expression and V(D)J immune receptor libraries were constructed and sequenced.

RESULTS: A total of 8362, 70785, and 8706 single cells from kidney biopsies, PBMC, and Treg ACT, respectively, passed QC filtering. Discrete Treg clusters were identifiable in both intervention and control biopsies through expression of FOXP3 and IKZF2. T cells followed by NK cells, monocytes, and B cells were the most abundant populations identified. The top 7 TCR clonotypes across all samples were seen exclusively in the patient receiving ACT and were present in both peripheral blood and the biopsy with significant clonal overlap (Morisita Index: 0.823).

CONCLUSIONS: We identify discrete Treg clusters within biopsies and peripheral blood. TCR repertoires between peripheral blood and kidney transplants demonstrated clear overlap which was higher in the patient receiving Treg ACT, suggesting infiltration of the allograft with adoptively transferred cells.

P.06 **The intrathecal compartmentalised, autoantigen-specific antibody-secreting cell dominated response in patients with autoimmune encephalitis**

Ruby Harrison[1], Jakob Theorell[1,2], Robyn Williams[1], Meng Zhao[1], Andrew Fower[1], Georgina Miller[1], Zoe Wu[1], Eleanor Browne[1], Bo Sun[1], Adam Handel[1,3], Mateusz Makuch[1], Sarosh Irani[1, 3]

[1]Oxford Autoimmune Neurology Group, Nuffield Department of Clinical Neurosciences, University of Oxford, UK; [2]Department of Clinical Neurosciences, Karolinska Institutet, Stockholm, Sweden; [3]Department of Neurology, John Radcliffe Hospital, Oxford University Hospitals, Oxford, UK

Autoantibodies against Leucine-rich glioma inactivated 1 (LGI1) and Contactin-associated protein-like 2 (CASPR2) are causative in patients with encephalopathies characterised by neuropsychiatric impairments and distinctive seizures. We aimed to study the B cell response in the cerebrospinal

fluid of these patients. Cerebrospinal fluid (CSF) B cells from three patients with active LGI1- or CASPR2-antibody encephalitis were single sorted and sequenced. Cells were selected for cloning monoclonal antibodies (mAbs) on the basis of whether they represented clonal expansions or singlets. Strikingly, 119/137 (87%) antibody secreting cells were LGI1/CASPR2 reactive, versus 4/16 (25%) B cells ($p<0.0001$). 9/30 (30%) mAbs which were unreactive with LGI1 or CASPR2 bound to either rodent neuronal cultures or rat brain sections, indicating as yet undiscovered brain autoantigens. Comparisons of clonality, light chain usage, IgG subclass, mutations and heavy chain CDR3 characteristics identified features which correlated with LGI1/CASPR2 reactivities and strength of binding, particularly clonality, IgG4 subclass, number of heavy chain mutations, and heavy chain CDR3 length. To ask if germline precursors of LGI1/CASPR2 reactive mutated mAbs retained binding, we removed mutations and observed 33/118 (28%) retained antigen binding. Our findings showed patients with LGI1/CASPR2-antibody encephalitis harbour a markedly enriched and diverse population of autoantigen-reactive antigen secreting cells in their CSF, many of which are germline encoded.

P.07 **Combining adoptive T-cell therapy with cancer vaccines to enhance anti-tumour immune responses**

Vinnycius Pereira Almeida, James McAuliffe, Laurine Noblecourt, Emily Steffke, Amanda Wicki, Silvia Panetti, Carol Leung, Benoit van den Eynde

Ludwig Institute for Cancer Research, University of Oxford, UK

Adoptive T-cell (ATC) therapy has been used in clinical trials to treat patients with solid tumours expressing MAGE, a shared cancer-associated antigen family. However, ATC alone used to treat solid tumours has resulted in unsatisfactory disease control in clinical trials. To exhibit an optimal effect, it is crucial for infused T cells to infiltrate the tumour microenvironment (TME), which is often immunosuppressive, especially in “cold tumours”. The chimpanzee adenovirus ChAdOx1 and the orthopoxvirus MVA vaccine vectors have been described to increase T cell infiltration in the tumour microenvironment. Therefore, the objective of this project was to investigate if these vaccines coding the P1A antigen (murine model of MAGE) can synergise with ATC therapy to reduce tumour burden and the possible mechanisms involved in such synergy. Tumour-bearing mice treated with ATC and vaccines were able to control the tumours and had long term protection and survival significantly higher than the treatments given alone. This control was correlated with a superior T-cell function, proportion and cell number of antigen-reactive T cells in different immune compartments. Further investigation of the antigen-presenting cells (APCs) after vaccination showed they present a higher activation and maturation status in comparison to non-vaccinated controls. In conclusion, combining ATC with viral vector vaccines led to a significant tumour control, which was correlated with increased APCs activation and T cell expansion. Single cell RNA sequencing experiments will be performed to better understand the impact of vaccines on the T cell transcription profiles.

P.08 **Are Fibroblasts Immune Cell Filters in Peripheral Tissues?**

Zhi Yi Wong[1], Ilya Korsunsky[2], Matthias Friedrich[1], Mathilde Pohin[1], Mark Coles[1] and Christopher D. Buckley[1]

[1]Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK
[2]Division of Rheumatology, Inflammation and Immunity, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

In rheumatoid arthritis, like in many chronic immune-mediated diseases, lymphocytes accumulate around blood vessels: a phenomenon termed 'perivascular cuffing'. The mechanisms driving this age-old histological observation remain obscure, but recent work that has addressed lymphocyte compartmentalisation within lymph nodes may hold a clue.

It has previously been shown that the GGT5-GGG-P2RY8 axis helps confine B cells and T follicular helper cells within germinal centres. Using multiple scRNA-seq datasets, we found that GGT5 expression is upregulated in inflammation, and is highly expressed by CCL19+ inflammatory fibroblasts, whereas P2RY8 is expressed by immune cells including monocytes, T and B cells. Multiplex high-dimensional imaging shows that GGT5+ fibroblasts and P2RY8+ leucocytes co-localise the same regions in inflamed tissues.

After co-culturing fibroblasts with PBMCs to mimic leucocyte infiltration, fibroblasts skew towards inflammatory phenotype whereby CCL19 and GGT5 expression increases. Depleting CD14+ cells abrogate this effect. Further investigations reveal that TNF potently stimulates GGT5 expression in fibroblasts and upregulates CXCL1 and CXCL2 expression.

During inflammation, tissue-resident leucocytes produce TNF which stimulates GGT5, CXCL1 and CXCL2 expression in fibroblasts. Later when infiltrating monocytes encounter fibroblasts, GGT5 and CCL19 expression is amplified. As GGT5 is also important in regulating leukotriene pathway that attracts eosinophils and neutrophils, we propose that GGT5+ fibroblasts attract multiple leucocyte subsets into tissues, but specifically use the GGT5-GGG-P2RY8 axis to restrain P2RY8+ leucocytes by restricting their migration. This mechanism prevents them from roaming freely in peripheral tissues by defining a confinement zone to enforce their accumulation in the perivascular space, causing perivascular cuffing.

Poster presentations

P.09 **Predisposition of blood group incompatibility and g6pd deficiency to severe neonatal hyperbilirunaemia**

Eugene J. B. Freiku[1], Kenneth K. desBordes[2], Francis Agyemang-Yeboah[3], Alex Osei-Akoto[4], Max E. Annani-Akollor[3], Eric Konadu[2]

[1]Laboratory Department, Briteaid Health Specialist Clinic, Kumasi, Ghana; [2]Department of Clinical Biochemistry, Komfo Anokye Teaching Hospital; [3]Department of Molecular Medicine, School of Medicine and Dentistry, KNUST; [4]Child Health Directorate, Komfo Anokye Teaching Hospital & School of Medicine and Dentistry, KNUST

Hyperbilirubinaemia occurs in infants of all racial groups. It is also seen in infants with bloodgroup incompatibilities, erythrocyte-enzyme deficiencies and also infants with structural defects of the erythrocytes. One of the major causes of neonatal jaundice is fetal-maternal blood group incompatibility involving the ABO system, the Kell system and the rhesus system. Our study found out that blood group incompatibility and G6PD deficiency are some of the haematological factors that influenced the development of hyperbilirubinaemia.

P.10 **Role of non-myocytes in hypertrophic cardiomyopathy, a common genetic heart muscle disorder**

Ying-Jie Wang[1], Kamayani Singh[1], Jose Coelho Lima Junior[5], Adam Lokman[2], Julia Beglov[1], Matthew Kelly[1], Andrew Blease[1], Sunitha Balaraju[1], Sahar Ghaffari[1], Lee-Anne Stork[3], Mahon Maguire[3], Jurgen Schneider[6], Mark Coles[4], Chris Buckley[4], Elizabeth Soilleux[5], Charles Redwood[1], Houman Ashrafian[1], Hugh Watkins[1]

[1]RDM Cardiovascular Medicine, University of Oxford, UK; [2]Department of Physiology, Anatomy & Genetics, University of Oxford, UK; [3]Department of Oncology, University of Oxford, UK; [4]The Kennedy Institute of Rheumatology, University of Oxford, UK; [5]Division of Cellular and Molecular Pathology, University of Cambridge, UK; [6]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK

Hypertrophic cardiomyopathy (HCM) is a common, serious, genetic heart muscle disorder. The biophysical mechanisms by which HCM mutations in sarcomeric proteins disrupt cardiomyocyte function are largely understood, but the cellular and molecular pathways leading on to the complex and adverse remodeling of the non-myocyte compartment are unexplained. Here we report that cardiac tissue from people with HCM exhibit a prominent chronic infiltration of immune cells. We found a similar infiltration in a translationally faithful HCM mouse model (Actc1E99K). RNA Seq data from human and mouse HCM hearts revealed a profound and active immune response. This immune reaction causatively contributes to pathogenesis of HCM as genetic depletion of lymphocytes (Rag-1-/-) in these mice led to profound adverse cardiac remodeling. Detailed longitudinal characterization of HCM hearts revealed that regulatory T cells (Tregs) were enriched and functionally activated in HCM. Both Treg transfer and in vivo Treg expansion significantly ameliorated cardiac remodeling in HCM. These data contribute to our understanding of HCM and support Tregs as a clinically testable novel therapeutic strategy in cardiac fibrosis.

P.11 **High Dimensional Immune Cell Profiling of Peripheral Blood in Lumbosacral Radiculopathy: A Pilot Study**

Shuaiwei Wang, Simon Rinaldi, Annina B. Schmid, Alexander J. Davies

Nuffield Department of Clinical Neurosciences, University of Oxford, UK

Preclinical models demonstrate the potential role of peripheral immune cells in the development and resolution of neuropathic pain after nerve injury. However, a detailed understanding of the cellular immune response to peripheral nerve injury in humans is lacking. Here we sought to profile

the variation in peripheral blood mononuclear cells (PBMCs) in the context of painful lumbosacral radiculopathy. We quantified the innate and adaptive immune cell populations in PBMCs by full-spectrum flow cytometry using a custom-designed 23-colour panel, with a focus on markers of cytotoxic immunity. Serum levels of inflammatory cytokines were analysed by U-plex assay. We observed a significant increase in the proportion of CD4+ helper T (Th) cells and regulatory T (Treg) cells in the radiculopathy group versus controls. Conversely, we observed a decrease in the proportion of monocytes, while there were no significant differences in total NK, CD8+ T, B cell and dendritic cell populations between groups. NK cells showed elevated levels of the cytotoxicity-related protein perforin, compared to healthy donors. We also observed an increasing trend in the granzyme B-producing CD8+ NKT cells. Serum levels of interferon gamma (IFN-g), interleukin 4 (IL-4) and IL-12p70 showed a decreasing trend in the patient cohort. This study serves as a foundation to the further immune cell profiling in peripheral nerve injury and neuropathic pain. Future work will examine the dynamic changes in cell populations post-surgery and their relationship to pain outcomes.

P.12 **Pleural effusion mediates immune suppression by upregulating PD-L1 on cancer cells and PD1 on CD4+ T cells**

Yu Zhao[1, 2], Nikolaos I Kanellakis[1, 2, 3], Zheqing Zhang[1], Yanchun Peng[1, 4], Tao Dong[1, 4], Najib M Rahman[1, 2, 3]

[1]Chinese Academy of Medical Sciences (CAMS) Oxford Institute, Nuffield Department of Medicine, University of Oxford, UK; [2]Laboratory of Pleural and Lung Cancer Translational Research, Nuffield Department of Medicine, University of Oxford, UK; [3]Oxford Centre for Respiratory Medicine, Churchill Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; [4]Medical Research Council (MRC) Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK

Introduction: Malignant pleural effusion (MPE) is a common incurable cancer complication, associated with high mortality. Despite the approval of immune checkpoint inhibitors, the immunosuppressive properties of MPE fluid on cancer and immune cells remain elusive.

Methods: To study the immunosuppressive effect of MPE fluid, patient derived MPE cell lines and bulk T cells from cone blood specimens were exposed to a range of pleural fluid from malignant and non-malignant effusion, R10 (RPMI supplemented with 10% FBS, for cancer cells), or commercial T cell medium (for T cells), and human serum. Cell proliferation, expression of PD-L1, MHC-I, and PD-1 were measured via flow cytometry. Interleukin-8 (IL-8) protein levels in culture media pre- and post-culture with cancer cells were measured.

Results: Cancer cells cultured in MPE fluid demonstrated similar growth rates compared to culture medium (R10). Exposure of cancer cells to pleural fluid (PF) upregulated PD-L1 and downregulated MHC-I protein expression. No differences were detected in T cell apoptosis when T cells were cultured in T cell medium or MPE fluid. CD4+ T cells upregulated PD-1 when exposed to PF independent of aetiology. No differences were detected in PD-1 expression of CD8+ T cells when exposed to PF or T cell medium. MPE fluids exhibited higher levels of IL-8 compared to non-MPE. Cancer cells secrete IL-8 independent of culture condition.

Conclusion: This study suggests PD-1/PD-L1 axis and IL-8 as possible important mediators of immunosuppression in MPE microenvironment and supports the idea to drain MPE fluid as early and complete as possible.

P.13 **P2X7 receptor mediated cytokine release from human microglia (monocyte-derived); implications for patients with TBI and other brain pro-inflammatory conditions**

Jamie Cowley[1, 2], Neale Harrison[1, 2], Ines M. Morano[2], Hujo Chan[2], Elizabeth Jinks[2], Fabiana Corsi-Zuelli[1, 3], Ashley Pegg[1], Andrew R. Stevens[4,5], Antonio Belli[4,5], Rachel Upthegrove[6], Catherine A. Brady[1,2], John Gordon[2], Omar Qureshi[1, 2], Nicholas M. Barnes[1, 5]

[1]Neuropharmacology Research Group, Institute of Clinical Sciences, University of Birmingham, UK; [2]Celentyx Ltd, Birmingham, UK; [3]Department of Neuroscience and Behaviour, Division of Psychiatry, University of São Paulo, Brazil; [4]Institute of Inflammation and Aging, University of Birmingham, UK; [5]NIHR SRMRC Research Team, Queen Elizabeth Hospital Birmingham, UK; [6]Institute for Mental Health, School of Psychology, University of Birmingham, UK

Despite traumatic brain injury (TBI) being the leading cause of death and disability in people in their first four decades of life, there are no pharmacological treatments approved for this indication. The excitatory P2X7 receptor, expressed predominantly by microglia in the brain, drives a neuroinflammatory response that may exacerbate brain trauma after the primary insult. Antagonism of the P2X7 receptor may therefore reduce the pathological consequences of TBI and other brain pro-inflammatory conditions. The present study investigated the ability of the P2X7 receptor to modulate cytokine release (e.g. IL-1β) from human microglia in vitro.

Human microglia (iMDM) were differentiated from human peripheral blood monocytes by culture in a cocktail of cytokines over 5-10 days. The immuno-phenotype of the iMDM versus the originating monocytes, and expression of the P2X7 receptor, were assessed by immuno-cytochemistry and demonstrated high expression of microglial phenotypic markers compared to the originating monocytes. Following priming of iMDM with LPS, the P2X7 receptor agonist, BzATP, evoked a concentration-dependent release of pro-inflammatory cytokines including IL-1β. The cytokine release evoked by BzATP, was prevented by selective P2X7 receptor antagonism with A-804598, inflammasome inhibition with MCC-950 and caspase-1 antagonism with VX-765 in a concentration-dependent manner. A similar profile was evident when the intracellular entry of the fluorescent dye, YO-PRO-1, was used to monitor activity of the P2X7 receptor pore.

The present results demonstrate the ability of a P2X7 receptor antagonist to suppress pro-inflammatory cytokine release from microglia suggesting that P2X7 receptor antagonists may improve the clinical outcomes for patients with TBI.

P.14 **The liver transcriptomic landscape differs between SPF and Germ Free Mice**

Amit Halkhoree[1], Dr Nicholas Ilott[1], Dr Claire Pearson[1], Dr Kelsey Jones[1], Alex Grannell[1], Dr Petar Scepanovic[2], Prof Dame Fiona Powrie[1]

[1] Kennedy Institute of Rheumatology, University of Oxford, UK; [2] F. Hoffmann-La Roche AG, Switzerland

The liver is richly connected to the gastrointestinal system through bile acid metabolism which is, in part, mediated by the intestinal microbiota. The extent of the microbial control of this enterohepatic circuitry is not clearly understood, yet a breakdown in homeostatic interactions could lead to liver disease. To explore potential regional homeostatic interactions, we compared liver transcriptional profiles between germ free (GF) (n=15) versus specific-pathogen-free (SPF) (n=15) mice. We found approximately 2200 (FDR $p<0.05$) differentially expressed genes between GF and SPF mouse liver. This revealed a significant upregulation of type 1 interferon response (IFN-I) and significant downregulation of TGF-beta signalling. These results suggest that liver IFN-I mediated processes are microbially controlled, which may impact on processes such as liver regeneration and response to viral infection. It also suggests that the microbiome state is correlated with TGF-beta expression, which requires further investigation to determine the functional impact. Further work is on-going to explore the microbially mediated connections between the ileum and the liver though ileal transcriptomics, metagenomics, and metabolomics.

P.15 **Discovery, Selection and Proof of Concept Evaluation of Novel TCR Selective Targets in Ewing Sarcoma**

Qinan Qian[1], Bass Hassan[1,2]

[1]Sir William Dunn School, University of Oxford, UK; [2]Oxford Molecular Pathology Institute, UK

Ewing Sarcoma (EwS) is a rare aggressive bone and soft tissue tumour in young adolescents with dismal outcomes for disseminated disease. The most prominent driver variant is EWSR1-FLI1 fusion oncogene with additional less frequent somatic variants. Different efforts are being investigated to treat EwS but there are currently no immunological targeting approaches that have been approved. Adoptive cell therapy (ACT) is one strategy, where receptors on lymphocytes are used to identify and target antigens on EwS cells. Here, I have utilised advances in whole genome sequencing informatics to identify expressed candidate neoantigens unique to EwS as a pre-requisite for T-cell receptor identification. The pVACfuse integrated platform was applied to over 130 EwS cases and cell lines with RNA sequencing. Both somatic variants and fusion gene breakpoint peptides were identified. In-silico HLA-typing was performed using Optitype (MHC Class I) and HLA-HD (MHC Class II), and predicted binding affinities were identified for all peptides and expressed MHC class I and MHC class II. High-affinity breakpoint peptides were identified mainly with binding to MHC Class II. The next step is to validate candidate peptides presented by specific MHCs on antigen-presenting dendritic cells (DCs) to identify activating T- cells from donor peripheral blood mononuclear cell (PBMC) lymphocytes. Subsequent single-cell T cell receptor (TCR) sequencing and functional analysis will define selective functional TCRs that can be the basis for proof-of-concept TCR-based immunotherapies that may be taken forward to translational human studies.

P.16 **Therapeutic antibody discovery targeting complex membrane proteins**

Elizabeth Allen, Lara Bavinton, Megan Ingham, Nathan Jones, David Knight, Adam Kupinski, Gen Labbe, Tom Mills, Dale Starkie, Dave Llewellyn and Joe Illingworth

DJS Antibodies

Monoclonal antibodies (mAbs) represent a growing number of licensed therapies, targeting indications ranging from infectious disease, inflammatory and immunological disorders to cancers. As of January 2023, of the 128 licensed therapeutic mAbs, around half target membrane proteins, almost exclusively with type-I single span transmembrane domain and a prominent extracellular domain. Complex membrane proteins such as G-protein-coupled receptors (GPCRs) and ion channels represent around 30-40% of druggable targets, however as they are technically challenging for antibody discovery often presenting limited epitope, they remain underserved by mAb therapy, with only two approved mAbs targeting GPCRs.

Our proprietary HEPTAD platform is a novel approach to antibody discovery with specific capabilities targeting transmembrane protein targets, which have previously been intractable to biologics drugs. Using HEPTAD, we generated novel antibodies which bind the extracellular surface of LPAR1. From these, we identified a first-in-class lysophosphatidic acid (LPA) receptor 1 (LPAR1) antagonist antibody, which is currently in investigational preclinical studies for the treatment of fibrotic diseases.

P.17 **Peptide specific natural killer cell receptors**

Malcolm J. W. Sim[1, 2], Paul Brennan[1], Katherine L. Wahl[1], Jinghua Lu[1], Sumati Rajagopalan[1], Peter D. Sun[1] and Eric O. Long[1]

[1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD, 20852, USA; [2]Centre for Immuno-Oncology, Nuffield Department of Medicine, University of Oxford, Headington, UK

The class I and II human leukocyte antigens (HLA) present short peptide antigens for immunosurveillance. T cell receptors (TCR) exhibit exquisite peptide-specificity, discriminating similar peptides and distinguishing ‘self’ from ‘non-self’ antigens presented by HLA molecules.

However, peptide-specificity may not be limited to TCRs as recent studies demonstrated that germline-encoded receptors expressed on natural killer (NK) cells also exhibit peptide-specific recognition of HLA molecules. These Peptide-Specific Natural Killer cell Receptors (PSNKR) include members of the Killer-cell Immunoglobulin-like Receptors (KIR), CD94:NKG2C and NKp44. The KIR family contains many highly similar receptors that bind seemingly analogous HLA-I ligands with comparable outcomes when functionally engaged on NK cells. However, association studies link specific KIR-HLA-I combinations with the numerous diseases, which requires identifying the salient features that differentiate one KIR-HLA-I interaction from another. Here, we performed systematic screens totaling over 3,500 specific interactions to determine the specificity of five KIR for peptides presented by four HLA-C ligands. Inhibitory KIR2DL1 was largely peptide sequence agnostic, binding approximately 60% of HLA-peptide complexes tested. Inhibitory KIR2DL2, KIR2DL3, and activating KIR2DS1 and KIR2DS4 bound only 10%, down to 1% of HLA-peptide complexes tested, respectively. Activating KIR2DS1, previously described as weak, bound strongly to HLA-C with high peptide sequence specificity. Our data revealed HLA-restricted peptide recognition by germ-line encoded NK receptors and imply that NK cell responses can be shaped by HLA-I bound immunopeptidomes in the context of disease or infection.

P.18 **Development of BAFF-R-specific chimeric antigen receptor T cells**

Mike B Barnkob[1,2], Mette Stæhr[1], Julia Heiselberg[1], Zelvera Ismetova Usheva[1], Kimmie B Christensen[1], Huw York-Colin[2], Lasse V Joergensen[1], Nanna S Mikkelsen[3], Mia Andersen[1], Laerke JB Brandt[1], Maria Ormhøj[1,4], Mette Elverdam[1], Christian Nielsen[1], Yaseelan Palarasah[4], Rasmus O Bak[3], Marco Fritzsche[2], Torben Barington[1]

[1]Centre for Cellular Immunotherapy of Haematological Cancer Odense (CITCO), Department of Clinical Immunology, Odense University Hospital, University of Southern Denmark, Odense, Denmark; [2]Kennedy Institute of Rheumatology, University of Oxford, UK; [3]Department of Biomedicine, Aarhus University, DK-8000 Aarhus, Denmark; [4]Department of Health Technology, Technical University of Denmark, Lyngby, Denmark; [5]Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

Chimeric antigen receptor (CAR) T cells which target CD19 are proving to be an efficient immunotherapy against B-cell leukemia and lymphomas. However, some patients relapse with CD19 negative cancer cells and there is thus a need for identifying other targets for CAR therapy.

BAFF receptor (BAFF-R) is a TNF receptor superfamily receptor, which plays an important role in B-cell development, activation and survival through its binding to the soluble ligand B-cell activating factor (BAFF), making BAFF-R an attractive target for CAR therapy.

We first examined BAFF-R expression in leukocytes and haematological cancers by scRNAseq and flow cytometry and find high expression in B-cell cancers including mantle-cell lymphoma, diffuse large B-cell lymphoma and Burkitt's lymphoma, but not multiple myeloma and myeloid cancers. We generated two monoclonal antibodies against BAFF-R and examined their reactivity against the human proteome and by using human tissue arrays. We next constructed different BAFF-R-specific CARs based on heavy and light chains of these antibodies. We show that one of our BAFF-R CARs bound with high specificity to BAFF-R, and by examining calcium flux, and NFkB, AP-1 and NFAT signaling, we show that T cells transduced with these novel CARs were efficiently activated. Importantly, BAFF-R-specific CARs performed similarly to a CD19-specific CAR in terms of T-cell proliferation, cytokine production and cytotoxic killing. In vivo testing with BAFF-R-specific CARs is ongoing.

Taken together, we have developed an efficient BAFF-R-specific CAR, with the potential to work on its own or in combinations with CD19-specific therapies.

P.19 **The role of genetic variation on sensitivity to Natural Killer cell-mediated cytotoxicity in HGSOC Cell Lines**

Melville Nyatondo[1,2], Christabel Boyles[1], Michael Gill[1], Tim Halim[1]

[1]Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, UK; [2]School of Medicine, Ulster University, UK

Ovarian cancer is the sixth-leading cause of cancer deaths in women worldwide, accounting for 207,252 deaths and 313,959 new cases each year. High-grade serous ovarian cancer (HGSOC) is the most common type accounting for 75% of epithelial ovarian cancers. Debulking surgery and platinum chemotherapy, such as carboplatin, are commonly used as first-line treatments. However, HGSOC continues to face clinical challenges as it is highly heterogenous and characterised by high genomic alterations and copy number instability. Approximately 96% of HGSOCs contain somatic p53 mutations and other frequent mutations, including BRCA (13%) and KRAS (<10%). These mutations affect the tumour microenvironment and immune cell infiltration.

This study was carried out as part of method development to characterises the sensitivity of HGSOC cell lines with differing mutations to Natural Killer (NK) cell-mediated cytotoxicity. We focused on HGSOC cell lines with p53, BRCA2 and KRAS mutations (Ovcar3, Peo1 and Kuramochi) using an in vitro killing assay to better understand how these mutations affect sensitivity to NK cell cytotoxicity and expression of NK cell ligands. Our experiments revealed that the HGSOC cell lines are resistant to NK killing and express variation in stimulating and inhibiting ligands, especially in MHC-1. However, there was no effect in percentage lysis from MHC-1 blocking antibody, except in Kuramochi, suggesting alternative mechanism of resistance to NK cell cytotoxicity.

P.20 **Investigating the immune response in ethnically diverse human lymph nodes upon vaccination**

Jacqueline H.Y Siu[1, 2], Sofia Coelho[3], Aime Palomeras[3], Daniel Opoka[4], Vinod Kumar[2], Brian Marsden[1, 2], Samantha Vanderslott[5], Donald Palmer[6], Hashem Koohy[7], Anita Milicic[8], Pontiano Kaleebu[4], Teresa Lambe[5], Mark Coles[2], Katrina Pollock[3, 5, 9], Calliope Dendrou[1] for the LEGACY Network

[1]Wellcome Centre for Human Genetics, University of Oxford, UK; [2]Kennedy Institute of Rheumatology, University of Oxford, UK; [3]NIHR Imperial Clinical Research Facility, UK; [4]Uganda Virus Research Institute, Uganda; [5]Oxford Vaccine Group (Department of Paediatrics), University of Oxford, UK; [6]Royal Veterinary College, University of London, UK; [7]MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK; [8]Jenner Institute, University of Oxford, UK; [9]Department of Infectious Diseases, Imperial College London, UK

Development of effective vaccines against global and emerging pathogens is complicated by interpersonal differences in vaccine responsiveness. This is further complicated by the fact that previous studies have traditionally focused on the immune response in peripheral blood samples rather than the draining lymph nodes (LNs) where the inflammatory and germinal centre responses are mounted. With these challenges in mind, the LEGACY Network was formed to create an ethnically representative single-cell atlas of LNs sampled by ultrasound-guided fine-needle aspirates (FNAs) before and after vaccination. FNAs is a new, tractable, and safe approach for human LN sampling; thus, providing a new avenue for assessing human vaccine efficacy in vivo.

In the first cohort, we recruited individuals with Black (East and West African) or Asian (South and South-East Asian) ancestry to participate (n = 13). LN FNA and PBMC were collected at baseline and around five days after an adjuvanted influenza vaccination (Fluad Quadrivalent with MF59 adjuvant). We developed robust clinical protocol, tissue sampling and processing pipelines to capture LN cells and PBMC for downstream 5' 10x single-cell RNA sequencing with surface protein expression, BCR and TCR immune receptor repertoires. Having collected both the ipsilateral and contralateral axillary

lymph nodes, we identified proportional subset differences upon vaccination relative to the side which the vaccine was given. Overall, we aim to highlight the use of FNAs to study in vivo human immune responses, and generate an open resource that provides a valuable reference map for elucidating the drivers of interpersonal differences in vaccine responses.

P.21 **Myeloid-derived suppressor cells (MDSC) as modulators of Pseudomonas aeruginosa lung infection**

Maëlys BORN-BONY[1], Bérengère VILLERET[1], Mélodie BOUTE[1], Clémence GAUDIN[1], Romé Voulhoux[2], Laurye Van Maele[3], Jean-Claude Sirard[3], Ignacio GARCIA-VERDUGO[1], Jean-Michel SALLENAVE[1]

[1]Université de Paris, INSERM 1152, Physiopathologie et épidémiologie des maladies respiratoires, Faculté de Médecine, Hôpital Bichat, F-75018 Paris, France; [2]LCB-UMR7283, CNRS, Aix Marseille Université, IMM, 13009, Marseille, France; [3]Univ. Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 - UMR9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France

Introduction
Pseudomonas aeruginosa (P.a) is an opportunistic bacteria with high antibiotic resistance, responsible for severe inflammation, irreversible lung damage, and respiratory insufficiency, eg in cystic fibrosis patients. Our laboratory has previously shown that adoptive transfer of genetically modified regulatory macrophages was protective in mice infected in the lung with P.a (Kheir et al, Mol. Ther. 2022), showing the importance of regulatory cells in the resolution of P.a. infection. We therefore chose here to investigate the role of myeloid-derived suppressor cells (MDSCs) in the context of lung P.a infection.

Methods
MDSCs were obtained by differentiation from C57Bl6 mice bone marrow in IL-6 and GM-CSF-containing media in a 5 day protocol. Splenocyte inhibition assays were performed to assess MDSCs inhibition capacity in standard or Co-star Transwell plates. MDSCs were either mock- or infected with different P.a. strains for 6 hours. Supernatants were analyzed for their lymphocytic inhibitory capacity in splenocyte proliferation assays.

Results
When infected with P.a (PAO1 strain) or with clinical strains, MDSC supernatants were shown to inhibit lymphocyte proliferation. However, the effect was lost with PAO1 mutants lacking the flagella (dFliC) or affected in their mobility (mot), but remained unchanged in TLR5 -/- and Myd88 -/- MDSCs.

To determine which MDSC effectors are responsible for lymphocyte inhibition, protein purification experiments and mass spectrometry of infected MDSC supernatants are currently being performed.

Conclusion
Our results suggest that P.a impact MDSCs function by increasing their inhibition capacity, which may be beneficial to control excessive maladaptive inflammation during lung infections.

P.22 **Solutions for Simultaneous Single Cell Transcriptome Analysis and Immune Receptor Profiling**

Singleron Biotechnologies

Singleron Biotechnologies, Gottfried-Hagen-Straße 60, 51105 Cologne, Germany

The adaptive immune system is composed of a diverse array of antigen binding T-cells and B-cells. The diversity in T-cell and B-cell receptor sequences are governed by the recombination and somatic hypermutation in the V (variable), D (diversity) and J (joining) gene segments. Advancements in multi-omics approaches at a single cell resolution has led to adapting the cutting-edge GEXSCOPE® microwell-based technology, to provide gene expression data combined with the T-cell or B-cell receptor sequences, including the sequence information on the complementarity determining region 3 (CDR3). The improved performance of our GEXSCOPE® Single Cell V(D)J Kit enables high pairing rate of immunoreceptors. For full length immunoreceptor single cell sequencing, Singleron offers the sCircle® Single Cell Full Length TCR or BCR Sequencing Library Kit, which enables full length

V(D)J region sequencing at a single cell level. Characterization of therapeutical antibodies or T cell therapies at a single cell level can now be conveniently performed on both, human and mouse immunoreceptors. Singleron's immune profiling kits, offer unique high-throughput single cell solutions for clonal analysis, antibody discovery, or developing CAR-T therapies, which can be used without the necessity of specialized equipment.

P.23 **CALHM6: a synaptic channel that facilitates macrophage-mediated natural killer cell activation**

Sara Danielli[1], Zhongming Ma[4], Eirini Pantazi[1], Amrendra Kumar[2,3], Benjamin Demarco[1], Fabian A Fischer[1], Usha Paudel[4], Jillian Weissenrieder[4], Robert J. Lee[4,5], Sebastian Joyce[2,3], J. Kevin Foskett[4,6] and Jelena S Bezbradica[1]

The Kennedy Institute of Rheumatology, University of Oxford, UK; [2]Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, Tennessee, USA; [3]Department of Pathology, Microbiology, & Immunology, Vanderbilt University Medical Center, Nashville, Tennessee, USA; [4] Department of Physiology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, USA; [5]Department of Otorhinolaryngology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [6]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Membrane ion channels of the calcium homeostasis modulator (CALHM) family promote cell-cell crosstalk at neuronal synapses via ATP release, where ATP acts as a neurotransmitter. CALHM6, the only CALHM highly expressed in immune cells, has been linked to induction of natural killer (NK) cell anti-tumour activity. Its mechanism of action and broader functions in the immune system are, however, unclear. Here we generated Calhm6-/- mice and report that CALHM6 is important for regulating the early innate control of Listeria monocytogenes infection in vivo. We find that CALHM6 is upregulated in macrophages by pathogen-derived signals, and that it relocates from intracellular compartment to the macrophage-NK cell synapse, facilitating ATP release and controlling the kinetics of NK cell activation. Anti-inflammatory cytokines terminate CALHM6 expression. CALHM6 forms an ion channel when expressed in the plasma membrane of Xenopus oocytes, where channel opening is controlled by a conserved acidic residue, E119, but CALHM6 is localized to intracellular compartments in mammalian cells. Our results contribute to understanding of neurotransmitter-like signal exchange between immune cells that fine-tunes the timing of innate immune responses.

P.24 **Changing adjuvanted vaccine formulation tailors protection against malaria through distinct immune mechanisms**

Sören Reinke[1], Eirini Pantazi[2], Romain Guyon[1], Joannah Fergusson[1], Alexandra Sanchez Martinez[1], Ahmed M. Salman[1], Anjum Aktar[2], Ekta Mukhopadhyay[1], Roland Ventura[3], Floriane Auderset[3], Patrice M. Dubois[3], Nicolas Collin[3], Adrian V S Hill[1], Jelena S Bezbradica[2], Anita Milicic[1]

[1]The Jenner Institute, Nuffield Department of Medicine, University of Oxford, UK; [2]Kennedy Institute of Rheumatology Research, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Medical Sciences Division, University of Oxford, UK; [3]Vaccine Formulation Institute, Rue du Champ-Blanchod 4, 1228 Plan-Les-Ouates, Switzerland

Adjuvanted protein vaccines offer high efficacy, yet most potent adjuvants are proprietary and not easily available to vaccine developers globally. New adjuvant formulations are being developed by the Vaccine Formulation Institute (VFI) in Switzerland for global open-access clinical use. As one of the most severe and deadliest infectious diseases, we selected a malaria mouse model to assess the potency and mechanisms of VFI adjuvants. In the context of our leading malaria vaccine R21, we characterised four adjuvants from the VFI portfolio: two liposomal (LQ and LMQ) and two squalene emulsion-based adjuvants (SQ and SMQ), containing QS-21 saponin (Q), and optionally a synthetic TLR4 agonist (M). Two vaccine formulations, R21/LMQ and R21/SQ offered remarkably high protection (80-100%), comparable to the current leading proprietary adjuvant Matrix-M. While peak antibody titres were not informative for vaccine efficacy, we found that induction of functionally

superior antibodies that can prevent hepatocyte invasion by Plasmodium parasites is key for protection. Although equally protective in vivo, LMQ and SQ tiggered different innate mechanisms in macrophages, with LMQ, but not SQ, activating the NLRP3 inflammasome and inducing several NF-κB targets. This was recapitulated systemically in vivo, resulting in divergent innate and ultimately adaptive responses, with R21/LMQ resulting in TH1-skewed and R21/SQ in TH2-skewed immune responses. Overall, we describe how modular changes in vaccine formulation can be deployed for customised, immune-status tailored, vaccine design.

P.25 **The role of mitochondrial antiviral signalling protein (MAVS) in innate immunity against bacterial infection**

Yuqing Long[1,4], Paul Klenerman[2,3], Timothy Hinks[4]

[1]Chinese Academy of Medical Sciences Oxford Institute (COI), University of Oxford, UK;
[2]Peter Medawar Building for Pathogen Research, University of Oxford, UK;
[3]Translational Gastroenterology Unit, Nuffield Department of Medicine, University of Oxford, UK;
[4]Respiratory Medicine Unit, Nuffield Department of Medicine, University of Oxford, UK

Introduction: Mitochondrial antiviral signalling protein (MAVS) is a 540-amino acid protein, mainly localized on the mitochondrial outer membrane. Numerous studies have shown that MAVS signalling plays a pivotal role in maintaining antiviral immunity and antifungal immunity. MAVS, acting as an adapter for activated RIG-I and /or MDA5, can induce robust type I/III IFN production, which are important for host defence against intracellular pathogens. MAVS deficiency abolishes the induction of IFNs and prevents the activation of NFκB and IRF3. MAVS has also been associated with airways diseases such as asthma in genetic association studies.

Material and Methods: To study the role of MAVS in antibacterial host defence, we infected the C57BL/6 and MAVS KO mice with non-typeable Haemophilus influenzae (NTHi): the dominant bacterial pathogen involved in airways diseases. At different time points, bacterial loads, weight loss, lung tissue pathology, inflammatory and immune response of these two mouse strains during infection were profiled.

Results: During NTHI infection, 1) The mRNA level and protein level of MAVS were significantly decreased in C57BL/6 mouse lungs; 2) 24 hours post-infection MAVS KO mice harboured significantly pulmonary higher bacterial loads than WT C57BL/6 mice, but C57BL/6 mice suffered more weight loss, suggesting a stronger early, innate immune response in WT C57BL/6 mice; 3) Type I/III IFN production was significantly reduced in C57BL/6 mice and MAVS KO mice. 4) The inflammatory response and immune cell profile of MAVS KO mice are distinct from the C57BL/6 mice.

Conclusion: These data reveal an important, novel role for MAVS in regulating early innate immunity against a major respiratory bacterial pathogen.

Key words: NTHI, MAVS, IFN, innate immunity, asthma

P.26 **DIVE IN to Immune Niches: Combining high-dimensional human tissue phenotyping and AI-based immune microenvironment analysis to identify and quantify immune cell niches**

Ananya Bhalla, Mathilde Pohin, Joannah Fergusson, Tom Thomas, Christopher Buckley, Mark Coles, Dylan Windell

Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK

Advances in multiplexed imaging have enabled simultaneous detection of multiple cellular markers at single-cell resolution using fluorescence-based antibody staining and mass cytometry methods. The Cell DIVE platform provides a powerful tool to investigate immune microenvironments and interactions of immune cells with stroma and vasculature in spatially preserved niches using whole-slide widefield immunofluorescence imaging. The multiplexing process involves cyclical rounds of antibody staining, image acquisition and fluorophore bleaching, allowing simultaneous visualisation of up to 60 markers on a single tissue section. Using the Cell DIVE platform, we have validated over

90 markers across 12 different normal and pathological human tissues, allowing rapid multiplexed imaging of broad immune panels across tissue sites. The Cell DIVE platform further provides automatic removal of background autofluorescence, enabling sensitive and reliable multi-marker visualisation for downstream single-cell analysis. This has enabled the identification of immune cell populations and their stromal niches within human lymph nodes and provided spatial context to microenvironment changes in response to vaccine adjuvants. We have developed a custom multiplexed image analysis pipeline that employs the open-source AI-based Mesmer algorithm for nuclear and cellular segmentation. Quality control metrics, including comparison with background staining, are used to appraise each marker. Following the generation of a cell by marker intensity matrix, downstream analysis becomes possible for cellular immunophenotyping, providing an end-to-end multiplexed imaging toolkit for immunology research. This approach has been used to stratify patients with inflammatory bowel diseases and rheumatoid arthritis based on the tissue composition and location of immune cells to inform therapeutic responses.

P.27 **Single-cell RNA sequencing reveals the presence of immature neutrophils in inflamed murine joints**

E. van Grinsven, M. Attar, K. Zec, I. Udalova

Kennedy Institute of Rheumatology, University of Oxford, UK

Classically, recruitment of immature neutrophils to the circulation has mostly been reported in response to acute inflammatory stimuli. Recent studies have reported that some of the rheumatic diseases, such as rheumatoid arthritis, SLE, and GCA vasculitis, exhibit an increase in low density neutrophils (LDNs) in the blood. We and others have shown that these LDNs partly have an immature phenotype. Resembling patient data, we observed a shift in blood neutrophil maturity in a mouse model of rheumatoid arthritis. We improved our dissection methods to characterize neutrophils resident in the knee joint with flow cytometry, while eliminating contamination from the adjacent bone marrow. These analyses allowed us to demonstrate that immature neutrophils were not only found in the blood but were also recruited in large numbers to the inflamed knee joint. Single cell RNA sequencing of the myeloid cells in the blood and joints confirmed the presence of neutrophils with an immature phenotype. Integration of these datasets followed by pseudotime analysis with the slingshot package resulted in three predicted lineages originating from the immature neutrophils. Interestingly, two of the lineages ended in clusters of mature neutrophils found only in the joint. These joint-specific clusters showed increased expression of pro-inflammatory mediators compared to clusters of mature neutrophils that were present in the joint as well as in the blood. We are also analysing cellular localisation by fluorescence microscopy. In conclusion, the inflammatory micro-environment in the knee joint may drive local maturation of immature neutrophils to mature neutrophils with a pro-inflammatory, tissue-resident phenotype.

P.28 **Antigen-specific IgG avidity following vaccination with IV BCG or ID MTBVAC is associated with superior protection from M.tb challenge in non-human primates**

Marco Polo Peralta Alvarez[1], Stephanie A. Harris[1], Andrew White[2], Sally Sharpe[2], Nacho Aguilo[3], Carlos Martin[3], Helen McShane[1], Rachel Tanner[1]

[1]The Jenner Institute, University of Oxford, UK; [2]Public Health England, Porton Down, Salisbury, UK [3] Grupo de Genetica de Micobacterias, Departamento de Microbiología y Medicina Preventiva, Facultad de Medicina, Universidad de Zaragoza

There is increasing evidence that antibodies may play a role in protection from tuberculosis (TB). We compared the PPD-specific post-vaccination antibody responses in two studies of non-human primates: one with three groups: intradermal, aerosol and intravenous (IV) routes; and another with ID BCG and MTBVAC. IV BCG and MTBVAC drove a superior antibody response in terms of magnitude, opsonisation, and avidity, compared to ID or aerosol BCG. For both studies PPD-specific IgG avidity correlated with protection from in vivo challenge and in-vitro mycobacterial growth

inhibition, suggesting antibody affinity maturation might constitute an important feature of protective antibodies.

P.29 **Modelling homologous recombination deficiency in prostate cancer**

JTW Kwon[1], E Murphy[2], KC Chan[1], S Afolaranmi[1], SM Phyu[1], RJ Bryant[2] and EE Parkes[1]

[1]Department of Oncology, University of Oxford, UK; [2]Nuffield Department of Surgical Sciences, University of Oxford, UK

Background
Approximately 5-13% of prostate cancer (PCa) cases harbour alterations in homologous recombination (HR) genes, which are typically associated with earlier onset of disease and poorer prognosis. Aberrations in HR drive genomic instability and subsequently inflammation through activation of the cGAS-STING pathway. However, there are currently no in vivo immunocompetent models exploring the biology of defective homology-directed repair. We developed an in vivo model of PCa to explore the effects of HR deficiency on the tumour microenvironment (TME).

Methods
Palb2KD in MyC-CaP cells was validated via immunoblotting, immunofluorescence imaging and cell cycle analysis. MyC-CaP cells were also injected subcutaneously into the flank of male FVB mice to assess tumorigenicity, tumour growth delay and immune composition, both in the absence and presence of fractionated radiotherapy.

Results
Palb2KD MyC-CaP cells demonstrated an increase in dsDNA breaks, cytoplasmic dsDNA and micronuclei formation compared to HR-proficient cells. Palb2KD MyC-CaP cells also exhibited S phase stacking and upregulation of cGAS-STING pathway components. Notably, reduced Palb2 expression in MyC-CaP tumours not only produced a significant growth delay in response to 3 x 5 Gy radiotherapy, but also a shift in CD4+:CD8+ T cell and M1:M2 macrophage ratios in the TME.

Conclusion
Abrogated HR repair promotes genomic instability and subsequent cGAS-STING pathway activation in MyC-CaP cells. Defective Palb2 expression sensitises MyC-CaP tumours to radiotherapy and gears the TME towards a more immunosuppressive phenotype. HR deficiency may therefore mediate therapy resistance in PCa by fostering chronic inflammation and dampening anti-tumour mechanisms.

P.30 **Mechanism of Antigen Recognition by T cells in context of TCR-targeted bispecifics**

Mateusz Kotowski[1,2], Mafalda Santos[1,2], Markus Korbel[3], Edward Jenkins[1,2], Daniel Heraghty[3], Mai T. Vuong[1,2], James McColl[3], Ben de Wet[4], Steven F. Lee[3], David Klenerman[3], Simon J. Davis[1,2]

[1]Radcliffe Department of Medicine, University of Oxford, UK; [2]MRC Human Immunology Unit, University of Oxford, UK; [3]Department of Chemistry, University of Cambridge, UK; [4]Immunocore Ltd

ImmTACs are first-in-class bispecific biologics used for the immunotherapy of cancer. They act by engaging tumour-associated peptide-MHCs and T-cell receptors (TCRs) via their constituent αβTCR and anti-CD3 scFv moieties, respectively. In contrast to other bispecifics, ImmTACs display exceptional sensitivity to antigen, but the mechanistic basis for their high potency is still under investigation.

Using functional assays, we show that there is an affinity window for optimal T cell activation. More compact, N-terminal formats of the ImmTAC show ~20-fold greater antigen sensitivity than the extended C-terminal counterparts. We find that the ability to recruit CD8 co-receptor to the bispecific-bound TCR contributes to high potency of N-ImmTACs. C-terminal ImmTACs are unable to co-opt CD8, but this feature does not fully explain their diminished potency. Artificial extension of peptide-MHC differentially impairs the potency of the N-terminal and C-terminal ImmTACs, implying that dimensions of TCR-targeted bispecifics are important to their sensitivity.

We identify bona fide sites of antigen discrimination using a supported lipid bilayer (SLB) system combined with total internal reflection fluorescence microscopy. At these sites, referred to as close-contacts we observe accumulation of CD58 and exclusion of CD45. These contacts persisted at both high and low, cancer-like antigen density conditions. Overall, this work sets out new principles for design of T cell redirecting immunotherapies and demonstrates that the early CD45-depleted microvillar contacts are sites of TCR triggering.

P.31 **The Mechanisms of Allergy to Fungal Bioaerosols in the Lung**

Emma-Jane Goode and Emma Marczylo

Toxicology Dept, UKHSA Chilton, UK

Allergic airways disease (AAD) is a collective term for respiratory disorders that can be exacerbated upon exposure to airborne allergens. The contribution of fungal allergens to the pathogenesis of AAD has become well established over recent years and can be present in the air we breathe as bioaerosols. Understanding the mechanisms allergy to fungal bioaerosols can help to better manage symptoms and conditions, reducing the burden of disease. We conducted a comprehensive review to collate the current understanding of mechanisms involved in the allergic response to fungal bioaerosols in airway epithelia. To be included in the final analysis, studies were compared to defined inclusion/exclusion criteria, relevant data was extracted, and each paper was assessed using a quality scoring tool. The search string provided 440 initial results with 61 studies selected for final analysis. 120 potential target mechanisms were identified within the selected studies and 17 were identified in more than 5 studies. The major pathways identified in allergic response include 1) a role for proteases and the activation of the PAR2 receptor, 2) release of IL33, 3) EGFR pathway and mucin expression, and 4) a skewing towards a Th2 profile promoting eosinophil recruitment. However, there was conflicting evidence when comparing results from individual studies. Thus, further research is required to better understand which fungi/fungal components drive the allergic response.

Funding: This work has been funded under the NIHR Health Protection Research Unit for Environmental Exposures and Health with Imperial College London

P.32 **Rebalancing immune checkpoints in rheumatoid arthritis by targeting the synovial microenvironment**

Linda Mies, Jean-Baptiste Richard, Christopher Buckley, Kim Midwood

Kennedy Institute of Rheumatology, NDORMS, University of Oxford, UK

PURPOSE: Tenascin-C (TNC) is an immune-regulatory extracellular matrix protein, that is upregulated in the synovium during the onset of rheumatoid arthritis (RA). High serum levels of TNC are associated with hard-to-treat, erosive disease and can predict patients whose pain will not improve with anti-TNF therapy, whilst synovial TNC levels decrease in people with spontaneously resolving synovitis. TNC blockade prevents disease progression and tissue destruction in experimental arthritis. The aim of this project is to better understand the role of TNC in human RA pathology.

METHODS: Single-cell RNA sequencing data was used to investigate the cellular source of TNC in the OA and RA synovium, and CellDIVE multiplexed imaging was used to determine the localization, and cellular targets, of TNC in synovial biopsies.

RESULTS: TNC is expressed exclusively by stromal cells in the synovium, particularly fibroblasts. Gene expression amongst fibroblast subsets changes with disease type and/or stage. Two distinct groups of RA patient can be defined by the presence or absence of TNC in the synovial lining-layer. Females are more likely to have high lining-layer TNC, and males more likely to have TNC-enriched lymphoid-aggregates. Regardless of patient demographic and tissue localization, TNC-rich niches are consistently populated with elevated macrophage numbers. Moreover, autoantibody-positive patients showed increased TNC+ B-cells in lymphoid-aggregates.

DISCUSSION: Synovial expression of TNC in RA is heterogenous and associated with distinct pathogenic cell neighbourhoods.

CONCLUSIONS: Differential expression of synovial extracellular matrix may contribute to the different disease aetiologies/treatment responses observed in male and female, or autoantibody positive and negative, RA.

P.33 **Histopathological and transcriptomic analysis of lung tissue from rhesus macaques involved in preclinical SARS-CoV-2 vaccine studies to characterise immune signatures associated with pathology**

Cillian Gartlan[1], Amy R Cross[2], Francisco J. Salguero[3], Tom Tipton[1], Joanna Hester[2], Fadi Issa[2], Miles W. Carroll[1]

[1]Wellcome Centre for Human Genetics and Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, UK; [2]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK; [3]Research and Evaluation, UK Health Security Agency, Porton Down, Salisbury, UK

A greater understanding of the tissue immune responses following SARS-CoV-2 vaccination and challenge is required. To address this, we examined lung tissue from rhesus macaques challenged with the same dose of virus and culled at the same timepoint. Two rhesus macaques from every vaccine group (DNA, mRNA, formalin-inactivated and viral vector vaccines), as well as two unvaccinated and two re-challenged macaques, were chosen for these analyses – one macaque with the highest in-group pathology score and one with a low pathology score.

Immunohistochemistry (CD3, CD20, CD68 and MPO) was carried out on lung tissue slides together with RNAScope for SARS-CoV-2 spike mRNA to define infected and immune-infiltrated areas. Immunohistochemistry identified significant associations between pathological score and CD3, CD20 and spike mRNA.

Given background staining and a low number of stained cells for CD68 and MPO respectively, a machine learning pipeline created in ARIVIS Vision4d was used to count macrophages and neutrophils across three lung tissue sections from each macaque. This revealed associations between particular groups and macrophage and neutrophil numbers. For instance, macrophage numbers were higher in formalin-inactivated vaccine recipients and neutrophils higher in re-challenged macaques.

Based on the areas defined above, pathology was further characterised using the Nanostring GeoMx digital spatial profiling platform. Four pathological and 3 non-pathological regions in lung tissue from macaques of interest were assed using a whole-transcriptome probe panel to quantify RNA transcripts in these regions. Data analysis is ongoing, with the aim of understanding how different vaccines modify the tissue-specific host response.

P.34 **Development and Preclinical Characterization of Bispecific Monoclonal Antibody (BiMAb) for Colorectal Cancer Immunotherapy**

Norah Alrishedan[1], Walter Bodmer[1], Viorica Liebe lastun[1], Ali Bransi[2], Pablo Umana[2], Christian Klein[2], Rita Mateus Seidl[2], Vita Golubovskaya[3], John Wu[3]

[1] Oncology Department/ Weatherall Institute of Molecular Medicine, Oxford University, UK; [2]Roche Glycart AG , Switzerland; [3]ProMab Biotechnologies, California USA

Immunotherapy using T cell-engaging bispecific monoclonal antibodies (BiMAb) is a promising cancer therapy. Such BiMAbs bind simultaneously to immune effector cells and to a cancer-specific antigen on tumor cells, resulting in killing of the latter.

Placental alkaline phosphatase (PLAP), a plasma membrane-bound glycoprotein, is one of the four members of alkaline phosphatase isozyme family. PLAP is encoded by the ALPP gene. PLAP is expressed in placenta and has not been detected in other normal tissues, though there is some cross reaction with testicular tissue. It has been shown that PLAP is released into the serum of patients

with PLAP expressing tumours such as testis tumours. When PLAP is expressed ectopically in cancers, such as ovarian or colon carcinomas, it is essentially cancer specific and so an excellent target for immune based antibody therapy. The focus of my work is on colorectal cancer (CRC) and will mainly be based on the use of a well characterised panel of over 100 colorectal cancer derived cell lines about 20% of which express PLAP at the mRNA and protein levels . The cell lines are good representatives of primary tumors to use for in vitro preclinical testing of a new immunotherapeutic PLAP x CD3 BiMAb being developed for treatment of CRC.

Worldwide, colorectal cancer has one of the highest cancer incidences and in the United States is the third cause of mortality in cancer patients. This emphasises the need to find novel effective treatments for colon cancer

We found that a CD3 x PLAP BiMAb induced specific killing of PLAP-positive colorectal cancer cell lines, using peripheral blood mononuclear cells (PBMCs) as source of T cells, and that the killing depends on PLAP expression. The expression of PLAP in our cells varies and there is heterogeneity of PLAP expression within cell lines. However, we found that the effect of CD3 x PLAP BiMAb treatment was extended to PLAP-negative cells, when co-cultured with PLAP-positive ones, indicating a bystander effect. The bystander effect on PLAP-negative cells is only visible after 48 hours of treatment, suggesting an indirect killing mechanism. It is important to study bystander killing because it is a mechanism for targeting cancer cells that escape direct immune targeted killing.

To investigate further the mechanism of bystander killing, our early findings suggest CD3 x PLAP BiMAb activated T cells are induced to kill non-target expressing neighbouring cells. We are now studying the role of T cell subtypes and of the soluble factors secreted by the activated T cells and other mononuclear cells in activated T bystander killing.

P.35 **Iron restricted CD8+ T-cells display metabolic perturbations and show sensitivity to aspartate supplementation**

Megan Teh[1], Joe Frost[1], Linda Sinclair[2], Nancy Gudgeon[3], Barbara Kronsteiner-Dobramysl[4], Jennie Roberts[3], Jan Rehwinkel[1], Susanna Dunachie[4], Sarah Dimeloe[3], Andrew Armitage[1], Hal Drakesmith[1]

[1]MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK; [2]Cell Signalling and Immunology, University of Dundee, Dundee, UK; [3]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK; [4]Centre for Tropical Medicine and Global Health, Peter Medawar Building for Pathogen Research, University of Oxford, UK

Iron-deficiency affects ~2 billion people, and ~2% of human genes encode iron-interacting proteins involved in processes including mitochondrial metabolism, epigenetic regulation and DNA synthesis. Low iron profoundly impairs T-cell immunity, but the mechanisms underlying this phenotype remain unclear.

We conducted transcriptomic and proteomic screens of iron-deficient murine CD8+ T-cells to identify aspects of biochemistry most impacted by iron restriction. T-cells cultured in iron depleted conditions showed suppression of genes involved in mTORC1 and MYC signalling, suggesting that iron scarcity may modify T-cell metabolism. Proteomic analysis also revealed upregulation of mitochondrial proteins involved in oxidative-phosphorylation and beta-oxidation. Combined with published data indicating that iron-restriction suppresses mitochondrial but not glycolytic ATP synthesis, we proposed that mitochondrial function may be particularly sensitive to iron-deficiency.

Iron starved T-cells showed depletion of the mitochondrial tricarboxylic acid (TCA) cycle metabolites, alpha-ketoglutarate and malate, the synthesis of which lies downstream of the iron-dependent enzymes, aconitase and succinate-dehydrogenase, respectively. Supplementation of aspartate, an essential TCA cycle product, substantially ameliorated the inhibitory effects of iron-deficiency on T-cell proliferation and effector function. Concurrently, iron restricted T-cells have elevated mitochondrial reactive oxygen species, suggestive of electron transport chain dysfunction. We

hypothesise that this effect may be driven by elevated levels of mitochondrial proteins in the absence of the necessary iron cofactors.

Our data indicates that iron restriction impairs mitochondrial function at multiple nodes, but that specific interventions can overcome sensitivity to iron-deficiency. Our work provides metabolic mechanisms that partially explain the inhibition of T-cell responses by iron deprivation.

P.36 **Identification and characterisation of Th17 subpopulations in colorectal cancer patients**

Declan Pang[1], Mathilde Pohin[1], Alice Bertocchi[1], Alina Janney[1], Nicholas Lim[2], Nicholas Ilott[1], Holm Uhlig[3], Fiona Powrie[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, UK; [2]Peter Medawar Building for Pathogen Research, University of Oxford, UK; [3]Nuffield Department of Medicine, University of Oxford, UK

Th17 cells are important members of the CD4+ T helper compartment and play a key role in protecting against pathogens. However, they have also been implicated in multiple autoimmune diseases and cancers, including colorectal cancer (CRC). Although previous studies have described substantial plasticity within Th17 cells, how different Th17 expression programmes contribute to tumour progression remains poorly understood.

Th17 cells were selected from a publicly-available single cell RNA-sequencing dataset and grouped using unsupervised Louvain clustering. Three transcriptionally distinct clusters were identified, resembling a stem-like memory (Th17scm), an activated (Th17act) and a tissue-resident memory (Th17rm) subpopulation. Each subpopulation was spatially enriched and their existence was validated ex vivo using flow cytometry. Gene ontology demonstrated that Th17act were more differentiated than Th17scm, whilst metabolic differences primarily discriminated between Th17rm and Th17 act. The enrichment of Th17rm cells in colorectal cancer patients based on bulk RNA-seq signature expression was associated with a more immune-activated tumour microenvironment and improved patient prognosis.

P.37 **Discordant role for germinal centres in adenovirus vector- and mRNA vaccine-induced antibody responses**

Lyn Yong[1], Claire Hutchings[2], Tom Tipton[3, 4], Miles Carroll[3, 4], Ellie (Eleanor) Barnes[1, 2, 5], Paul Klenerman* [1, 2, 4], Nicholas M. Provine* [1, 4]
*co-senior

[1]Translational Gastroenterology Unit, University of Oxford, UK; [2]Peter Medawar Building for Pathogen Research, Nuffield Department of Clinical Medicine, University of Oxford, UK; [3]Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, UK [4] Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, UK; [5] NIHR Oxford Biomedical Research Centre, University of Oxford, UK

Anti-TNF therapy is used as front-line immunosuppressive treatment for several inflammatory diseases. Patients on anti-TNF therapy have impaired antibody responses to COVID-19 vaccines, recording lower titres and reduced durability of spike-specific antibodies. We sought to elucidate mechanistically how anti-TNF therapy modulates the humoral responses induced by the novel COVID-19 vaccines: mRNA-1273 (nucleoside-modified mRNA) and AZD1222 (replication incompetent adenovirus vector).

Impaired induction of germinal centre (GC) B cells (Fas+PNA+IgD-) was observed following vaccination with both platforms in multiple TNF signalling-deficient mouse models: double receptor knockout (Tnfrsf1a-/-Tnfrsf1b-/-), single receptor knockout (Tnfrsf1a-/-), TNF knockout (Tnf-/-) and TNF-inhibition with monoclonal antibody (α-TNF). Consistent with the central role for GCs in the development of antibodies, mRNA-1273 vaccination of these mice resulted in lower anti-spike antibody titres and reduced spike-specific B cell (IgD-S1+) responses.

In contrast, while there was attenuation in GC B cell frequency following AZD1222 vaccination – to varying degrees across the anti-TNF models – antibody titres at early timepoints post-immunisation were indistinguishable from wildtype mice. Antibody titres correlated with neutralisation capacity, suggesting the utilisation of alternative pathways to produce effective antibodies following AZD1222 immunisation. Interestingly, the Tnfrsf1a-/-Tnfrsf1b-/- mice recorded lower spike-specific B cells which was concordant with GC B cells, but this was not observed in Tnfrsf1a-/- mice, highlighting further discordance between GCs and spike-specific B cells in response to this vaccine.

Collectively, the results highlight fundamental differences in the regulation of humoral immune responses between the two vaccines, and suggest that AZD1222 can induce effective humoral immunity while bypassing the microanatomic germinal centre structure.

P.38 **Lipid presentation by CD1c in cancer**

Rita Szoke-Kovacs[1,2], Richard Suckling[1], Robert Simmons[1], Daniel Fonseca[1], Mariolina Salio[1]

[1]Immunocore Ltd; [2] Molecular Cellular and Immune Biology Doctoral School, University of Debrecen

Cytotoxic T cells are important participants in the body's antitumor immune response. These cells have been proved to be effective in targeted cancer therapy, however their application is limited by allotype restriction caused by the extreme polymorphism of “classical” Major Histocompatibility Complex (MHC) molecules. Whereas the “classical” MHC molecules present tumour-associated peptide antigens for specific T cell recognition, the “non-classical” MHC-like molecules such as the Cluster of Differentiation 1 (CD1) molecules, belonging to the non-polymorphic MHC class Ib group, can present microbial lipids and self-lipids to specific T cells. The CD1 type c (CD1c) molecules were described to present lipid molecules that predominantly accumulate in leukaemia cells, so specific CD1c restricted T cells can mediate the killing of these leukaemia cells. Several other malignant cell types also express CD1c molecules, therefore these molecules can be regarded as targetable tumour markers. The non-polymorphic nature of these molecules renders them ideal therapeutic target for patients of different genetic background. Whether the CD1c molecules are present in all types of leukaemia and the mechanism of CD1c recognition by T cells is currently unknown, thus the medicinal potential of these molecules remains limited. In this study, we analysed the lipid antigen repertoire associated with soluble CD1c complexes to get a better understanding of the lipid presentation by these molecules. Our data supports the view that the CD1c molecules accommodate most lipids from the cell’s lipidome. Looking for possible cancer specific lipid antigens, we aim to dissect the lipid antigen repertoire associated with CD1c complexes in cancer cells. We also aim to investigate different blood born malignancies for the presence of CD1c and determine how it is recognised by T-cells. These findings may allow the development of CD1c-targeting reagents specific to these malignancies and suitable across the whole human population by overcoming genetic restriction.

P.39 **Mapping vaccine adjuvants in space, time and cells**

Eloise Nee[1], Joannah Fergusson[1,2], Sören Reinke[2], Calliope Dendrou[3], Anita Milicic[2], Mark Coles[1]

[1]Kennedy Institute Of Rheumatology, University of Oxford, UK; [2]Jenner institute, University of Oxford, UK; [3]Welcome Centre for Human Genomics, University of Oxford, UK

While vaccine adjuvants are widely used, in both pandemic and non-pandemic contexts, their mechanisms of action are not well explored. Previous work has shown that two adjuvants, a liposomal based adjuvant (LMQ) and a squalene emulsion based adjuvant (SQ) are equally protective against a malaria challenge in mice but by different mechanisms. In this work we used fluorescently labelled versions of these adjuvants to map their distribution across organs, within tissues and cells. Additionally, their mechanisms of action are dissected at the cellular level by single cell transcriptome sequencing of the early inflammatory response. By mapping adjuvant cell tropism, we find that liposomal adjuvants target lymph node stromal cells in vivo and induce inflammation in cultured fibroblasts, whereas squalene emulsions do not. Single cell RNA sequencing further reveals inflammation programmes In both myeloid and stromal cell populations. Ultimately, we aim to

understand how targeting of resident lymph node cells, such as fibroblasts, programs the resultant adaptive immune response.

P.40 **Investigating the impact of iron limitation on regulatory T cell function in transplantation**

Marie Sion[1], Amy Cross[1], Joe Frost[2,3], Sarah Wideman[2], Joanna Hester[1], Fadi Issa[1], Alexander Drakesmith[2]

[1]Translational Research Immunology Group, NDS, Oxford, UK; [2]Weatherall Institute of Molecular Medicine, Oxford, UK; [3]Memorial Sloan Kettering Cancer Centre, New York USA

Introduction: Cellular therapies have the potential to facilitate immunosuppression reduction after transplantation. Of these, regulatory T cells (Tregs), a suppressive subset of T cells that maintain tolerance, are promising candidates. Iron deficiency has been shown to impair T and B cell responses, which could be beneficial in prolonging graft survival. Hypoferremia may affect Tregs differentially from conventional T cells (Tconvs) given their distinct metabolic profiles.

Methods: Mice receiving fully mismatched skin allografts were treated with low iron diet and a hepcidin mimetic to lower serum iron. Mice bearing the Tfrc(Y20H/Y20H) mutation, which impairs the transferrin receptor and leads to inefficient cellular iron import, were studied in both transplantation and malaria infection models. To investigate cell-intrinsic hypoferremia, adoptively transferred Tfrc(Y20H/Y20H) Tregs and Tconvs in immunodeficient transplant recipients were used to study in vivo suppression and rejection, respectively. Mixed chimeras using Tfrc(Y20H/Y20H) bone marrow allowed further study of iron-impaired immune development and alloresponses.

Results: Hepcidin treatment impaired alloresponses but only prolonged graft survival when combined with low iron diet. Tregs appeared more resistant to hepcidin than Tconvs and maintained activation markers more readily than Tconvs in transplant, infection, and chimera models, as well as in vitro. In all models, the Treg:Tconv ratio shifted in favour of Tregs in iron deficiency. However, while adoptively transferred Tfrc(Y20H/Y20H) Tconvs were incapable of rejecting in vivo, neither were Tfrc(Y20H/Y20H) Tregs capable of suppressing.

Conclusions: Hypoferremia can shift the Treg:Tconv ratio and promote tolerance by exploiting preferential Treg resistance to iron deprivation, which has therapeutic potential.

P.41 **An ex vivo model of inflammation in humans; lymph node responses to vaccine adjuvant**

Joannah Fergusson[1,2,3], Jacqueline Siu[1,2], Eloise Nee[1], Ananya Bhalla[1], Soren Reinke[3], Calliope Dendrou[2], Mark Coles[1], Anita Milicic[3]

[1]Kennedy Institute for Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK; [2]Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, UK; [3]The Jenner Institute, Nuffield Department of Medicine, University of Oxford, UK

Our understanding of the fundamental mechanisms underpinning human immunological processes are largely based on studies from mice, due in part to their ease of manipulation and tissue collection, which are then directly translated to humans. However, numerous pathway differences are likely to exist in these evolutionarily distant species. Complementary studies in humans generally rely on isolation of cells from tissues, with loss of valuable information about tissue organisation and non-cellular components.

We have developed a novel ex vivo system for studying immunological pathways within intact human lymphoid tissue. Healthy human lymph nodes, the hub of immune response generation, are cut into 300uM cross-sections, thus maintaining tissue architecture and extracellular matrix components. The tissue slices remain viable and functionally responsive to perturbations, such as stimulation with vaccine adjuvants, in culture. Combining this system with single cell RNA sequencing, we are dissecting the physiological mechanisms underlying the induction of inflammation in humans, and discovering how these differ from those induced in pre-clinical animal models. We reveal a potent activation of NK cells and a role for stromal populations in neutrophil

recruitment in response to adjuvants. Further, through the use of multiplexed widefield immunofluorescence imaging, we can identify immune microenvironments established in spatially preserved niches upon adjuvant stimulation.

This ex vivo model enables pathways to be elucidated which were previously difficult to study in humans, revealing new mechanisms of action for clinically relevant adjuvants, and with the potential to challenge paradigms of immune function established from studies in mice.

P.42 **LARP1 regulates macrophage metabolism and polarisation in the immune microenvironment of ovarian cancer**

Fenella Gross[1], James Chettle[1], Eirini Pantazi[2], Zinaida Dedeic[1], Molly Browne[1], Leticia Campo[1], Alistair Easton[1], Jelena Bezbradica Mirkovic[2], Sarah Blagden[1]

[1]Department of Oncology, University of Oxford, UK; [2]Kennedy Institute of Rheumatology, University of Oxford, UK

Cancers are known to establish protumoural, immunosuppressive microenvironments to drive immune evasion. Mechanisms to avoid immune detection and killing can include restricting antigen recognition, repolarising tumour-associated macrophages (TAMs) and inducing T cell exhaustion. Immune evasion is also thought to be essential in allowing pre-invasive tumours to progress to invasive cancer. For instance, high grade serous ovarian cancer (HGSOC) is usually preceded by pre-invasive lesions, known as serous tubal intraepithelial carcinomas (STICs), that remain latent for 5-7 years. We have shown by multiplex immunohistochemistry, that immunosuppressive M2-like macrophages cluster around STICs and that T cells fail to infiltrate the growing STIC. While the majority of tumour cells strongly express the oncogenic RNA-binding protein LARP1, we were surprised to also observe high LARP1 expression in both macrophages and T cells. Mechanistically, we have shown that LARP1 regulates the metabolism of both cancer cells and macrophages through direct binding of mRNAs. As macrophage and T cell phenotype is known to be strongly regulated by an altered metabolic microenvironment, we investigated how this was able to influence macrophage function. We performed RNA-Seq on primary macrophages from multiple human donors and demonstrated the ability of LARP1 to regulate network-level gene expression. We found that expression of classic 'M2-like markers' such as IL10 and CD206, were reduced in primary macrophages following LARP1 depletion. Together, these findings indicate that LARP1 has both an intrinsic and paracrine effect on the polarisation of stromal TAMs and may drive the immunosuppressive precancerous niche in STICs and in HGSOC.

P.43 **Understanding signalling from immune checkpoint in T cells**

Sumana Sharma[1], Toby Whitehead[1], Matheuz Kotowski[1], Edward Jenkins[1], Emily Ng[1], Judith Leitner [2], Jessica Soo Weei Ng[1], Yi-Ling Chen[1], Peter Steinberger[2], Simon J Davis[1]

[1]RDM Investigative Medicine, MRC-WIMM, University of Oxford, UK; [2]Institute of Immunology, Medical University of Vienna, Vienna, Austria

The cellular signalling process is a complex mechanism involving multiple players. In recent years, genetic screening using the CRISPR-Cas9 system has been adopted in order to uncover the cellular components involved in a wide range of contexts. Here we study the mechanisms by which inhibitory protein, PD1, expressed on the surface of T cells mediate their function using both genome-scale and focused arrayed CRISPR-screening approaches. Using a cell model, we systematically knockout ~60 SH2-domain containing proteins known to be important for T-cell signalling downstream of the T-cell receptor and the costimulatory molecule CD28 and study how the inhibitory signalling from PD1 upon engagement with its native binding partners, PDL1 modulate the activatory signalling. In a genome-wide knockout screen, we identify the previously uncharacterised mechanisms by which cytoskeletal proteins modulate activatory signalling. The outcome of this work will help better elucidate the mechanisms by which inhibitory molecules work thereby improving therapies based on their use in the context of cancer and autoimmunity.

P.44 **Preclinical models for the assessment of vaccine efficacy against hazard group 4 pathogens**

Stuart Dowall, Linda Easterbrook, Stephen Findlay-Wilson, Emma Kennedy, Lucy Flett and Roger Hewson

UK Health Security Agency, UK

Emerging viral pathogens continue to cause major public health concerns to human populations. To rapidly be able to respond to these threats, we need to understand the disease pathogenesis, the natural progression of infection and host response. For this purpose, in vivo models have been developed, which can answer the above concerns but in addition have value in assessment of intervention strategies including vaccine development.

We have established animal models for several viral haemorrhagic fevers caused by hazard group 4 pathogens including Crimean-Congo Haemorrhagic Fever, Nipah, Lassa, Marburg and Ebola viruses. For some models the virus has required host adaptation to increase disease severity whereas for others transgenic mice have been utilised. Due to the requirement of containment level 4 laboratories for the handling of these live pathogens, the facilities at the UK Health Security Agency are one of only a few available capable for assessing vaccine efficacy with live challenge virus.

These models have proven their value in testing the efficacy of vaccine candidates being developed, including differentiating the best constructs and supporting the progression of promising approaches into human clinical trials. Given the epidemic potential for many of these pathogens, and their inclusion as priority pathogens by many strategic groups (e.g. WHO, CEPI, UK Vaccine Network), these models are available for testing and will be refined according to the pathogen landscape.

P.45 **Discovery, pharmacophore characterisation and in vitro molecular evolution of anti-chemokine peptides**

Serena Vales[1], Jhanna Kryukova[1], Soumyanetra Chandra[1], Gintare Smagurauskaite[1], Megan Payne[1], Charlie J. Clark[1], Katrin Hafner[1], Philomena Mburu[1], Graham Davies[1], Garrett M. Morris[2] and Shoumo Bhattacharya[1]

[1]Wellcome Centre for Human Genetics, Radcliffe Department of Medicine, University of Oxford, UK; [2]Department of Statistics, University of Oxford, UK

CC and CXC-chemokines are the primary drivers of leucocyte migration in inflammatory disease, but redundancy in the chemokine network thwarts pharmacological intervention. Tick evasins promiscuously bind multiple CC and CXC-chemokines, overcoming redundancy. Here we show that hexadecapeptides that promiscuously bind both chemokine classes can be identified from evasins by phage-display screening performed with multiple chemokines in parallel. We identify two conserved motifs within these peptides and show using saturation-mutagenesis-phage-display and chemotaxis studies that an anionic patch in the first motif and hydrophobic, aromatic and cysteine residues in the second are necessary for function. We show that three different docking methods, AlphaFold2-Multimer, AutoDock CrankPep and CABS-Dock, agree in their predictions that the peptides occlude distinct receptor-binding regions in CC and in CXC-chemokines. We show that saturation-mutagenesis-phage-display also identifies mutations enhancing binding and inhibition of CC and CXC-chemokines and combinatorial-mutagenesis-phage-display identified mutation combinations that further enhance binding. Our results indicate that peptides with broad-spectrum anti-chemokine activity and therapeutic potential may be identified from evasins, the pharmacophore characterised by saturation-mutagenesis-phage display and computational modelling, and binding profile improved by in vitro molecular evolution. This pipeline could be applied to other disease-causing protein interaction networks.

P.46 **Mapping T Cell Epitopes in SARS-CoV-2 Variants of Concern**

Sandra Adele, Vishal Rao, Isanawidya Paramita, Anucha Preechanukul, Priyanka Abraham, Isabel Neale, Barbara Kronsteiner, Carl-Philipp Hackstein, Paul Klenerman, Susanna Dunachie, Eleanor Barnes

Peter Medawar Building for Pathogen Research, Oxford, UK

Background
Many Omicron descendent lineages have recently emerged. We characterised the magnitude, breadth and escape of T-cell recognition to Omicron variants of concern (VOC; BA.2/BA.4/BA.5) in COVID-19 vaccinated volunteers.

Methods
T-cells targeting wildtype (WT) epitopes were identified in 17 triple vaccinated volunteers, using PBMCs stimulated with 178 spike SARS-CoV-2 peptides, in a minipool-matrix peptide strategy, in IFN-γ ELISpot assays. Following the identification of WT antigenic-targets, T-cells were clonally expanded using WT single peptides and IL-2 (14 days), then IFN-γ/TNF/IL2 measured was following restimulation with WT and matched VOC peptides in Intracellular cytokine assays (ICS).

Results
All volunteers generated T-cells targeting >1 WT peptide (median 6, range 2-21). In all, 86 distinct antigenic targets were identified, predominantly located in spike NTD (31%) and RBD (26%). 56/86 were conserved between WT and VOC; however, 6/18 NTD and 13/23 RBD targets contained mutations in BA.2/BA.4/BA.5 relative to WT. T-cells targeting 23 WT peptides, identified in the ELISpot assays, were maintained following expansion and stimulation with VOC peptides in ICS. Loss of cytokine secretion was seen in 18 antigenic targets, whilst an increase was seen in 13 using VOC in ICS. Interestingly, gain in function was only seen in those previously infected with omicron.

Conclusions
Most spike specific T-cells target antigens that are conserved between WT and recent omicron VOC lineages. However, loss of T cell recognition to some VOC antigens is observed when evaluated at the single peptide level. Ongoing monitoring of T-cell escape as pandemic lineages evolves is critical to understanding COVID-19 immunity.

P.47 **Big Autofluorescent Lipid-Laden Macrophages Regulate Adrenal Corticosteroid Output**

Conan J O O'Brien[1], Aleksander Stawiarski[1], Giorgio Ratti[1], Emma Haberman[1], Yusuke Mizuno[2], Tomohiro Ishii[2], Tomonobu Hasegawa[2], David R Greaves[3], Siamon Gordon[3], Ana I Domingos[1]

[1]Department of Physiology, Anatomy, & Genetics, University of Oxford, UK;
[2]Department of Paediatrics, Keio University of Medicine, Tokyo, Japan;
[3]Dunn School of Pathology, University of Oxford, UK

The adrenal glands are hormone secreting glands that sit on top of the kidneys. Adrenal glands produces glucocorticoids, mineralocorticoids, and catecholamines, and are critical regulators of the stress response, metabolism, and blood pressure. Despite being identified for more that 30 years, our understanding of adrenal macrophages is incomplete . In numerous other tissues macrophages carry out a plethora of physiological and homeostatic roles, in addition to their classical immune functions. Herein, we describe a subset of lipid-laden adrenal macrophages that accumulate in a particular layer of the gland, increasing in number and size with age in male mice. Furthermore, we present data suggesting these foamy-like macrophages accumulate certain metabolites and thereby regulate adrenal hormonal output. We hereby provide novel insights into the physiological roles of macrophages in the adrenal gland and the mechanisms by which adrenal hormone release is regulated.

P.48 **Molecular determinants underpinning MAIT cells self-reactivity**

Robert Simmons, Vijayvkumar Karuppiah, Rory Crean[*], Richard Suckling, Miriam Hock, Marc Van Der Kamp[**] and Mariolina Salio.

Immunocore Ltd, Milton Park, Abingdon, Oxon, UK, [*]Uppsala University, Department of Chemistry, Husargatan 3, 752 37 Uppsala and [**]University of Bristol, School of Biochemistry, Bristol, UK

A remarkable characteristic of unconventional T cells is self-reactivity, which has been shown to shape basal reactivity of myeloid cells but might be deleterious in amplifying autoimmune responses driven by peptide specific T cells. Herein, we characterise a self-reactive MR1-restricted MAIT T cell receptor (E8 TCR), which we identified from a library of TCRs cloned from Vα7.2+ CD161+ MAIT cells. In cellular assays, the E8 TCR recognises MR1 molecules irrespective of the ligand loaded, as well as empty MR1 K43A molecules. The E8 TCR binds to all the tested MR1-ligand complexes with similar low micromolar affinity (KD =0.002-0.6 μM), as measured by biophysical assays using surface plasmon resonance. Co-crystals of this TCR with seven different MR1-antigen complexes reveal a similar docking mode to that previously reported for the canonical MAIT TCR AF7. Additionally, we identify a prominent role of the TCR residue R96β, which contacts MR1 residues E76 and E149. Molecular dynamics studies confirmed the relevance of this TCR-CDR3β loop in conferring self-reactivity. Interestingly, a significant increase in the use of this CDR3β motif is observed amongst self-reactive MAIT cell clones, as reported by our collaborators at the University of Basel (Chancellor and De Libero). This work furthers our molecular understanding of MR1-antigen complexes discrimination, identifying a ligand-agnostic mode of recognition.

P.49 **Engineering T cells with enhanced ligand discriminatory powers**

Jose Cabezas-Caballero, Mikhail Kutuzov, Anton van der Merwe, Omer Dushek

Sir William Dunn School of Pathology, University of Oxford, UK

Background: Adoptive T cell transfer is a promising type of immunotherapy. T cells are engineered ex-vivo to express T cell receptors (TCRs) that target specific tumour antigens. However, the exogenous TCR has not undergone thymic selection in the patient and therefore, can exhibit cross-reactivity to self-antigens. A clinical trial using an engineered TCR (a3a) resulted in fatal toxicity that has now been traced to cross-reactivity to a lower-affinity self-antigen (Titin). There is an urgent need to identify methods to prevent T cell activation to lower-affinity peptides whilst maintaining a potent response to higher-affinity target peptides.

Methods: The c259 TCR is an affinity-matured receptor that recognises the NY-ESO-1 cancer-testis antigen. Here, we produced a panel of eight peptides that differ in potency to the c259 TCR and measured their binding affinities at 37°C using surface plasmon resonance. Next, we transduced human primary T cells with the c259 TCR and measured their activation against cancer cells pulsed with a titration of the 8 peptides with different affinities. Finally, we screened the role of different T cell surface receptors and intracellular signalling molecules on T cell ligand discrimination.

Results: We have identified multiple targets that regulate T cell ligand sensitivity. Interestingly, we have also identified targets that decrease sensitivity to low-affinity peptides, without reducing sensitivity to the high-affinity on-target peptide.

Conclusions: Our findings show that T cells can be engineered to exhibit enhanced ligand discrimination. This approach could be applied to minimise the risk of lethal cross-reactivities in T cell therapies.

P.50 **Exploring the specificity of γδ Tumour Infiltrating Lymphocytes**

Alina Popa, Lauren Chessum, Shaliny Ramachandran, Thao Huynh, Kafayat Ololade, Andrew Preston, Mark Middletonb* Christopher Hollanda.

[a]Immunocore Ltd., 92 Park Drive, Abingdon, Oxfordshire, UK; [b]Department of Oncology, University of Oxford, UK

The T lymphocyte arm of the adaptive immune system recognises infected or transformed cells via the T Cell Receptor (TCR). T cells are divided into two subtypes based on whether they express TCR ab or gd . TCRs of the abtype target peptides presented on the plasma membrane of cells in the context of Human Leucocyte Antigen (HLA) molecules.

The Immunocore ImmTAX platform uses genetic engineering to create soluble αβ TCRs against peptide-HLA targets specifically expressed on tumour cells, infected cells or in the context autoimmune diseases. The number of patients that can be treated using the ImmTAX platform is currently limited by HLA diversity (i.e., polymorphism).

Immunocore works to overcome limitations of HLA-restricted therapies by exploring putative cancer ligands for T cells bearing the gd TCR. T(gd) cells can infiltrate tumours and have demonstrated ability to kill cancer cells, which may lead to favourable clinical outcomes for some types of malignancy. Although incompletely characterized, cancer cell ligands targeted by gd T cells are predominantly non-polymorphic and if targetable, may widen patient cohort size currently restricted by HLA molecules. The current research aims to investigate tumour cell specificity of the gd TCR repertoire found infiltrating digestive tract tumours that may be useful in expanding the versatility of ImmTAX.

P.51 **A high affinity soluble MAIT TCR as a tool to monitor MR1 antigen presentation**

Richard Suckling, Carole Perot, Robert Simmons, Robert Pengelly, Vijaykumar Karuppiah, Daniel Fonseca, Mariolina Salio

Immunocore Ltd, Milton Park, Abingdon, Oxon, OX14 4RY, UK

The non-polymorphic ubiquitously expressed MHC class I-related molecule, MR1 presents small metabolite intermediates of the vitamin B2 biosynthetic pathway, present in several bacterial and fungal species. MR1 is predominantly retained within the ER, and requires ligand loading prior to trafficking to the cell surface, which limits our detection of cell surface MR1 with currently available antibodies. Mucosal-associated invariant T (MAIT) cells recognise microbially derived vitamin B-related metabolites presented by MR1, and therefore have an important role in controlling infection. Considering that both commensal and pathogenic microbes have the capacity to generate MR1 ligands, and that MR1 molecules are ubiquitously expressed, it remains to be determined whether MAIT cells, which are highly abundant at mucosal sites, are continuously recognising their cognate antigen. To monitor MR1 ligand display at steady state and changes upon infection, we generated a high affinity version of a canonical human MAIT TCR, which can be used as a highly sensitive staining reagent. We report on the characterisation of this TCR, its specificity and its suitability to track MR1-antigen expression in vitro, in human and murine cell lines.

P.52 **The divergent localisation and trafficking of the pre-TCR is signalling independent**

Sam J. Garforth, Andrei I. Smid, John R. James

Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK

Expression of the pre-T cell receptor (pre-TCR), a complex of pTα and a recombined TCRβ chain, is an important checkpoint during αβT cell development. The preTCR complex is known to be only transiently expressed and rapidly internalised in developing T cells, and is thought to signal in an autonomous, ligand-independent manner.

However, these unique features of the pre-TCR compared to the final TCR complex also hinder its biochemical characterisation. Whilst previous studies have investigated the trafficking of the pre-TCR, identifying a mechanistic basis for the divergent function of this receptor has been confounded by the concomitant signalling that is normally present. To overcome this constraint, we have reconstituted pre-TCR complex expression in non-immune cells to directly uncouple the trafficking dynamics of the receptor from its associated signalling.

We find that all the defining features of the preTCR, including its lysosomal targeting are intrinsic properties of the receptor itself and are not the consequence of ligand-independent receptor activation. We demonstrate that it is the exposure of a hydrophobic region on the TCRβ chain that drives its trafficking and not any part of the unique pTα sequence per se.

Finally, we provide evidence that transitory pre-TCR cell surface expression can initiate weak tonic signalling in the absence of a ligand, suggesting how the preTCR can drive αβTCR lineage commitment.

P.53 **Development of Bivalent vaccines targeting Ebola and Sudan, or Marburg and Lassa viruses: pre-clinical immunogenicity and efficacy and early clinical safety and immunogenicity**

Amy Flaxman[1], Sarah Sebastian[2], Rebecca Makinson[3], Sofia Appelberg[4], Kuan M Cha[5], Marta Ulaszewska[6], Jyothi Purushotham[7], Ciaran Gilbride[8], Hannah Sharpe[9], Helen Sanders[1], Daniel Jenkin ([6]), Paola Cicconi, Abigail Platt ([3]), Nguyen Tran ([3]), Syona Neeraj ([3]), Alison Lawrie ([3]), Ian Poulton[3], Alexandra J Spencer[10], Stuart Dowall[11], Sue Charlton[11], Sarah Gilbert[6], Ali Mirazimi[4] & Teresa Lambe[1]

[1]Oxford Vaccine Group, Dept of Paediatrics, University of Oxford, UK; [2]Vaccitech plc, Harwell, UK; [3]Jenner Institute, Nuffield Department of Medicine, University of Oxford, UK; [4]Folkhälsomyndigheten, Public Health Agency of Sweden, Solna, Sweden; [5]R-BIOPHARM AUSTRALIA PTY LTD, New South Wales, Australia; [6]Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, UK; [7]Scripps Research Institute, La Jolla, CA, USA; [8]Baseimmune, London, UK; [9]University of Pennsylvania, Philadelphia, USA; [10]University of Newcastle, Australia; [11]UK Health Security Agency, Porton Down, Salisbury, UK

Viral haemorrhagic fevers (VHF) pose a significant threat to human health. In recent years, VHF outbreaks caused by Ebola, Marburg and Lassa viruses have caused substantial morbidity and mortality in West and Central Africa. In 2022, an Ebola disease outbreak in Uganda caused by Sudan Ebolavirus resulted in 164 cases with 55 deaths. There are no clearly defined correlates of protection against these VHF, impeding targeted subunit vaccine development. Any vaccine developed should therefore induce strong and preferably long-lasting humoral as well as cellular immunity against these viruses. Ideally this immunity should also cross-protect against viral variants, which are known to circulate in animal reservoirs. We used a viral vectored vaccine platform, ChAdOx1, to develop two Bivalent vaccines; one targeting Ebola and Sudan ebolaviruses and one targeting Marburg and Lassa viruses. ChAdOx1 technology has consistently demonstrated the capability to induce robust cellular and humoral antigen-specific immunity in humans, most recently in the Oxford-AstraZeneca ChAdOx1 nCoV-19 vaccine. We show here that our vaccines are immunogenic in mice, inducing strong cellular and humoral immunity post-immunisation. Importantly, we show that our BiEBOV vaccine (targeting Ebola and Sudan ebolaviruses) confers protection in a lethal ebolavirus challenge in both guinea pigs and mice. This vaccine has now entered phase I clinical studies. Both dose escalation and administering a booster dose have resulted in good safety and humoral immunogenicity profiles. BiEBOV is a strong candidate for deployment should another Ebola or Sudan ebolavirus outbreak occur in the coming years.

P.54 **The early emergence of aquaporin 4-specific B cells characterises neuromyelitis optica spectrum disorders**

Halwan Bayuangga[1,2], Sudarshini Ramanathan[1,3], Deniz Akdeniz[1], Min Gyu Im[1], Anne-Kathrin Kienzler[1], Ruby Harrison[1], Bo Sun[1], Akiko Nagaishi[1], Andrew Fower[1], Nicholas Yapp[1], Mark Woodhall[1], Nick Willcox[1], Mateusz Makuch[1], Russell C Dale[2], Fabienne Brilot[2], Lynn Dustin ([4]), M Isabel Leite[1,5], Jacqueline Palace[1,5], Daniel Anthony[6], Patrick J Waters[1], Sarosh R Irani[1,5]

[1]Oxford Autoimmune Neurology Group, Nuffield Department of Clinical Neurosciences, University of Oxford, UK; [2]Department of Neurology, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University, Yogyakarta, Indonesia; [3]Translational Neuroimmunology Group, Kids Neuroscience Centre, Children's Hospital at Westmead, Sidney, Australia; [4]Viral Infection and Immunity, Kennedy Institute of Rheumatology, University of Oxford, UK; [5]Department of Neurology, John Radcliffe Hospital, Oxford University Hospitals, UK; [6]Experimental Neuropathology Group, Department of Pharmacology, University of Oxford, UK

IgGs to aquaporin-4 (AQP4) are pathogenic in patients with neuromyelitis optica spectrum disorders (NMOSD), causing brain and spinal cord lesions. We aimed to understand how early AQP4-specificities might emerge during B cell development. PBMCs from 4 NMOSD patients and 2 healthy controls were single sorted from new emigrant, mature naïve, and memory B cell populations, representing their developmental progression, and differentiated into antibody secreting cells in vitro. Supernatants were tested for AQP4 reactivity and, from B cell transcripts, monoclonal antibodies (mAbs) were generated and characterised. Overall, only 2/7680 (~0.03%) B cells from healthy controls were AQP4-specific versus 36/14,304 (~0.25%, $p<0.0001$) from NMOSD patients which comprised ~0.7% new emigrant, ~0.1% mature naïve, and ~0.1% memory B cells. BCRs of AQP4-specific new emigrants and mature naïves were unmutated and antigen-reactivity could be retained across multiple heavy-light chain pairs. By contrast, in the memory BCRs, the highly mutated heavy chains were accompanied by a strict requirement for cognate pairing to recognise AQP4. All expressed memory BCRs retained binding to AQP4 upon reversion to their germline precursors (unmutated common ancestor; UCA). Upon injections into rodent brains, supplemented by human complement, mutated AQP4 mAbs generated human NMOSD-like lesions with pronounced loss of AQP4 and GFAP reactivity. This pathogenicity was reduced in the UCAs and absent from new emigrant or mature naïve mAbs. Our findings demonstrate break in central tolerance is principally responsible for emergence of AQP4-reactivities in NMOSD patients and their subsequent and precise maturation confers additional pathogenicity.

P.55 **Delineating the causal role of the gut microbiome in experimental colitis**

Rebecca Jeffery[1], Lilian Lam[1], Claire Pearson[1], India Brough[1], Elizete Araujo[1], Andrew Macpherson[2] Fiona Powrie[1] and Nicholas Ilott[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, UK; [2]University of Bern, Switzerland

Maladapted interactions between the mucosal immune system and gut microbiota are implicated in the pathogenesis of inflammatory bowel disease (IBD). Alterations in gut microbial communities are often seen in IBD patients, although the functional consequences of these shifts are not understood. Multiple mouse models of IBD fail to develop pathology when housed in germ-free conditions, suggesting an active role for the microbiota in disease pathogenesis. Helicobacter hepaticus is a murine pathobiont that induces colitis in interleukin-10 (IL-10) deficient mice, thus encompassing a role for both host and microbial factors in disease pathogenesis. Interestingly, IL-10 deficient mice monocolonized with H. hepaticus do not develop disease, suggesting other commensals are required to facilitate H. hepaticus-induced colitis. We have established that gnotobiotic mice stably colonized with the 12-member Oligo-Mouse-Microbiota (OMM12) are susceptible to H. hepaticus-induced colitis, facilitating the interrogation of commensal involvement in colitis development. Utilising longitudinal approaches, we have determined that the development of H. hepaticus-induced inflammation coincides with OMM12 dysbiosis, including an expansion of Enterococcus faecalis and reduction of Enterocloster clostridioformis and Akkermansia muciniphila, and alterations in

intestinal metabolite composition. Through monocolonization experiments, we have determined that E. clostridioformis supports H. hepaticus-induced colitis, an ability that is not shared by A. muciniphila, E. faecalis, Lactobacillus reuteri and Clostridium innocuum. Work is ongoing to understand the differential abilities of OMM12 members to support H. hepaticus-induced colitis. Ultimately, this work will identify microbiota interactions that promote colitis representing novel therapeutic strategies.

P.56 **The Microbiome and Airway Cytokines in Upper And Lower Airways in Severe Asthma**

M Jabeen[1], E Marchi[1], N Sanderson[1], C Barber[2], LCK Lau[2], ID Pavord[1], A Chauhan[3], P Klenerman[4], T Street[1], PH Howarth[2], TSC Hinks[1]

[1]NIHR Oxford Biomedical Research Centre and Respiratory Medicine Unit, The John Radcliffe Hospital, Oxford, UK; [2]Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Sir Henry Wellcome Laboratories and NIHR Southampton Respiratory Biomedical Research Unit, Southampton University; [3]Portsmouth Hospitals NHS Trust, UK; [4]Peter Medawar Building for Pathogen Research and Translational Gastroenterology Unit, Nuffield Department of Clinical Medicine, University of Oxford, UK

Introduction: Severe asthma is complex and immunologically heterogenous. Airways infection and innate immune dysregulation may confer inhaled corticosteroid resistance and remain clinically challenging. We hypothesise there are marked airway microbiome compositional shifts in severe asthma representing a 'treatable trait', with dominance of pathogenic species, warranting targeted therapy. In addition, these changes are thought to be distinct in the paucibacillary lower airways vs. the heavily colonised nasopharynx.

Methods: We performed Oxford Nanopore metagenomic sequencing, and RT-qPCR of induced sputum (n=97), and nasal lavage (NL;n=28 paired with sputum) samples from the Wessex Severe Asthma Cohort and Oxford Airways Study encompassing mild-moderate/severe asthma and health. Findings were integrated with clinical data and cytokine levels.

Results: A dominant pathogenic organism (H.influenzae, Streptococcus pneumoniae or Moraxella catarrhalis) was identified in sputum of 20% of patients with severe asthma and accompanied by sputum neutrophilia and elevated type-1 cytokines (including IL-1b, IL-6, IL-8, TNF; $p<0.01$, unpaired t-test, Benjamini-Hochberg correction). H. influenzae emerged as the most commonly isolated sputum pathogen in stable disease and is associated with elevated airway TNF and IL-10. Metagenomic analysis of NL demonstrated a distinct microbiome; presence of pathogenic organisms in the upper airways did not predict concurrent presence in sputum of severe asthmatics. In addition, sputum and nasal lavage cytokine measures correlate poorly within individuals.

Conclusion: The microbiome and cytokine milieu in the upper and lower airways are distinct. Airways infection is reliably identified in sputum at species level using long read metagenomic sequencing and associated with neutrophilic inflammation.

P.57 **Updated Therapeutic Antibody Profiling Contextualises the Developability of Human Lambda Antibodies**

Matthew I. J. Raybould, Oliver Turnbull, Annabel Suter, Charlotte M. Deane

Oxford Protein Informatics Group, Department of Statistics, University of Oxford, UK

Lambda light chain antibodies ('λ-antibodies') comprise 35% of natural human immunoglobulins but only around 10% of clinical-stage therapeutic antibodies (CSTs). Since λ-antibodies have distinct binding properties compared to kappa light chain antibodies ('κ-antibodies'), this bias constrains the diversity of epitopes that can be targeted by antibody drugs. There is growing evidence that λ-antibodies fail more frequently than κ-antibodies to advance through pre-clinical development, with hydrophobicity-driven aggregation being the primary concern.

Here, we use a computational approach to investigate developability-related contributions to the paucity of therapeutic λ-antibodies. We analysed c. 80,000 natively paired human antibodies and

compare them to c. 664 CSTs using our Therapeutic Antibody Profiler software, which calculates five descriptors related to poor developability across the predicted 3D structure. We update the original TAP pipeline with the latest deep learning-based antibody structure prediction tool, ABodyBuilder2.

Our analysis shows that λ-antibodies harbour larger patches of surface hydrophobicity (PSH) scores relative to κ-antibodies, supporting the increased aggregation risk of λ-antibodies over κ-antibodies. Our results also highlight a population of human λ-antibodies that lie at more moderate PSH values, suggesting that λ-antibodies as a class should not be ignored during development. We analysed atomic contributions to high λ-antibody PSH scores, demonstrating they are predominantly driven by the CDRL3, allowing us to propose rational engineering strategies.

Our work is the first time that deep learning-based structure prediction has been applied in the context of developability assessment. Our findings on λ-antibodies have direct applications in future screening library design, candidate selection, and lead optimisation.

P.58 **A single B-cell method to study the immunobiology underlying CASPR2-autoantibody encephalitis**

Sofija Paneva[1,2], Adam Handel[1], Ray Owens[3], Meng Zhao[1], Mateusz Makuch[1], Bo Sun[1], Sarosh R Irani[1]

[1]Oxford Autoimmune Neurology Group, Nuffield Department of Clinical Neurosciences, University of Oxford, UK; [2]Department of Pharmacology, University of Oxford, Oxford, UK; [3]Division of Structural Biology. Nuffield Department of Medicine, University of Oxford, UK

Antibodies to the neuronal surface protein CASPR2 are associated with CASPR2-autoantibody encephalitis, characterised by cognitive deficits and seizures. Patient sera and CSF predominantly target the discoidin domain of CASPR2. The underlying immunobiology is mostly unresolved, owing in part to the lack of sufficiently precise studies focusing on the antigen-specific B-cells.

To address this, we isolated PBMCs from four patients with CASPR2-autoantibody encephalitis and healthy controls (HCs). We used cross-lineage B cell surface markers including a fluorescently-tagged CASPR2 multimer to enrich for CASPR2-reactive B-cells, later differentiated into antibody-secreting-cells in vitro.

In total we isolated 535 CASPR2-specific B-cells, split equally across patients and HCs. CASPR2-specific B-cells from HCs were within the naïve B-cell compartment, whereas in patients they were within both naïve and memory compartments. Culture supernatants revealed that the EGF1/FibC and L1 domains of CASPR2 were most frequently targeted in HCs. In patients, the discoidin domain was targeted only by memory B-cells, while the remaining domains were targeted by both memory and naïve B-cells. Dimensionality reduction PCA on select FACS markers followed by UMAP analysis revealed that these discoidin-specific cells also formed a distinct population.

Sanger sequencing was used to retrieve and recombine cognate heavy and light chains of the B-cell receptors (BCRs). Further sequence analysis will be used to compare BCR sequence characteristics that distinguish CASPR2-reactive vs non-reactive B-cells in patients and healthy controls.

Our work promises to provide high-resolution understanding of how BCR sequence architecture relates to B-cell phenotype and precise epitope binding within this antibody mediated neurological condition.

P.60 **Genetic contribution towards TCR repertoire**

Esther Ng[1], Orion Tong[2], Robert Watson[2], James Gilchrist[2], Ben Fairfax[2], Yang Luo[1],

[1]Kennedy Institute for Rheumatology, University of Oxford, UK; [2]Weatherall Institute for Molecular Medicine, University of Oxford, UK

Background

The T cell receptor repertoire plays an important role in cancer, autoimmune disease and infection. However, little is known about the extent to which the usage of different V genes in the TCR repertoire is shaped by host genetics, especially in the context of cancer and Immune Checkpoint

Blockade (ICB). This study aims to investigate whether we can establish a genetic basis for TCR repertoire in CD8 T cells in a cohort of patients with melanoma and study interactions with treatment status.

Methods
Genotyping and TCR repertoire sequencing of CD8 T cells isolated from peripheral blood of patients with melanoma (n=199) receiving ICB treatment. A linear regression model was fitted between V gene usage and genotyping or HLA allele integrating age, gender and CMV status as covariates.

Results
Associations were identified at GWAS significance at chromosomes 14 (TRBV) and 7 (TRAV), cis to the respective genes. The peak associations being rs4725599 upstream of TRBV for TRBV-28 usage (P=2.0 x 10 -21) and rs7148819 associated with TRAV26-2 usage (P=2.0 x 10 -10). Independent associations were identified with HLA alleles including HLA C 07:02 with TRBV-5-6 (P=1.4 x 10 -5) and HLA B13 with TRAV26-1 (P=8.3 x 10 -5).

Conclusions
Our observations demonstrate a complex relationship between genetic variation and TCR repertoire usage in patients undergoing treatment with checkpoint immunotherapy. Given TCR usage is associated with immunotherapy responses, these observations have potentially important implications with respect to oncological outcomes.

P.61 **The embryonic origins of human finger joints reveals a cellular and anatomical basis for arthritis pathology**

Sarah Davidson[1], Davide Simone[1], Kathrin Jansen[1], Caio McHaido[1], Ian Reekie[1], Ananya Bhalla[1], Rowie Borst[1], Cesar Prada Medina[1], Zhi Wong[1], Nora Schwingen[1], Elena Lopez[1], Dylan Windell[1], Moustafa Attar[1], Catherine Disney[2], Andrew Bodey[2], Sharif Ahmed[2], Peter Lee[3], Stephen Sansom[1], Christopher D Buckley[1], Mark Coles[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, UK; [2]Diamond Light Source, Harwell Science & Innovation Campus, Didcot, UK; [3]UCL, Department of Mechanical Engineering, UCL, UK

Despite significant advances in our understanding of the cellular composition of Rheumatoid and Osteoarthritis, why particular joints develop different forms of disease remains an enigma. An important example includes the pattern of finger joint involvement. Both distal (DIP) and proximal interphalangeal (PIP) joints are affected in mechanical forms of arthritis, such as osteoarthritis (OA). However, PIP but not DIP joints, develop Rheumatoid arthritis (RA), an immune mediated inflammatory disorder. Functionally and spatially distinct populations of synovial fibroblasts have been shown to play a critical role in RA, driving inflammation and joint damage. Single cell sequencing of human foetal DIP and PIP joints, provide evidence for two discrete fibroblast lineages, derived from either a chondrogenic or mesenchymal origin. Our findings suggest that differences in abundance of these lineages, between joint locations, may contribute to the propensity of RA to affect proximal joints. Thus, cellular networks established during foetal development, may influence the discrete pattern of joint involvement in arthritis.

P.62 **Investigating the role of ZIP7 in modulating peripheral B cell function**

Jie Yan[1,2], Mukta Deobagkar-Lele[1], Tanya L. Crockford[1], Katherine R. Bull[1], Richard J. Cornall[1,2]

[1]MRC Human Immunology Unit, Nuffield Department of Medicine, University of Oxford, UK; [2]Chinese Academy of Medical Science Oxford Institute, University of Oxford, UK

Zinc is an essential biological trace element and a catalytic/structural component of a large variety of metalloenzymes and transcription factors. Our group reported a novel human agammaglobulinemia syndrome caused by hypomorphic mutations in a zinc transporter gene - ZIP7. However, how ZIP7 modulates peripheral B cell development and function is unknown.

To investigate the exclusive effect of Zip7 deficiency on peripheral B cells or leucocytes without disturbing their normal early-stage development, we developed a Zip7 P198A/flox Cd21-Cre model

and Zip7 flox/flox ERT2-Cre model, respectively, to control Zip7 knockout spatially and temporally. We then compared their phenotypes and responses after immunisation.

Mice with Zip7 P198A/- mature B cells had exclusively impaired pools of peripheral B-cell subsets. They presented an immunodeficiency phenotype due to defective generation of germinal centre B cells and marginal zone B cells, associated with a failure to produce antibodies against T-cell independent or T-cell dependent antigens. This immunodeficiency was not because early B cell activation events or metabolic pathways were disturbed. Even though the basal levels of peripheral cell subtypes were comparable in the tamoxifen-induced Zip7 knockout model, a significant decrease in producing antigen-specific germinal centre B cells was also shown.

Thus, Zip7 deficiency impairs the capability of producing germinal centre B cells and their functions. Our findings based on various qualitative and qualitative alterations of Zip7 indicate its finely tuned B cell-intrinsic effects for adaptive immunity.

P.63 **The role of costimulatory risk genes for polygenic inflammatory disease in CD4+ T cells**

Chloe Moscrop[1], Alexandru-Ioan Voda[1], Kristina Correa[1], Jonathan Hamp[1], Stephanie B. Hatch[2], Daniel Ebner[2], Holm H. Uhlig[3], Michael Dustin[1], Luke Jostins-Dean[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, UK; [2]Nuffield Department of Medicine, University of Oxford, UK; [3]Translational Gastroenterology Unit and Biomedical Research Centre, University of Oxford, UK

GWAS studies have successfully identified over 240 genetic risk variants for Inflammatory Bowel Disease (IBD), however many are in non-coding regions of the genome and their role in IBD pathogenesis remains largely unknown. Many of these risk variants are mapped to costimulatory receptors and are enriched in CD4+ T cells with classical CD28 costimulation. However, as many IBD risk variants map to alternative costimulatory proteins, are these studies potentially missing associations by only focusing on CD28 costimulation? Here, we are investigating CD4+ T high-throughput genetic and phenotypic assays to profile candidate genetic pathways implicated in IBD.

We will initially present data (n=5 healthy donors) investigating candidate risk pathways in CD4+ T cells costimulated by CD28 or alternative costimulation molecules ICOS, CD6 and CD27 using bulk RNA and ATAC -seq. We demonstrate that most pathways are activated by all conditions, but some key pathways depend on costimulation. For example, effector functions like proliferation and cytokine secretion were upregulated in CD28 costimulation whereas metabolic pathways such as oxidative phosphorylation were upregulated in alternative costimulation. We will next present first data from samples run on the high-throughput, semi-automated pipeline including genetic and transcriptomic analysis, cell surface and secreted proteomics, cellular function assays such as autophagy and immune synapse formation, combined with patient clinical data. The final data set will consist of 400 genotyped IBD patients and healthy individuals. Finally, we will outline the computational pipeline we have developed to analyse this large data set, including automated image analysis and expression QTL mapping.

P.64 **Objective quantification of neutrophil maturation by live 3d imaging of nuclear morphology**

J. Salafranca[1], Z. Ai[1], S. Alber [2], K. Ko[1], N. Karedla[1], E. van Grinsven[1], M. Fritzsche[1], I. A. Udalova[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom;
[2]University of Berkeley, Berkeley, California, United States

Recent studies have identified the heterogeneity in the neutrophil compartment as an important marker of inflammatory disease and cancer. Advanced technologies such as transcriptomics or mass cytometry have been used to characterise neutrophil subsets based on their molecular signatures. In clinical diagnostics neutrophils and their maturation stage have been largely characterised by nuclear morphology, using manual counting of 2D colorimetric microscopy images. However, this approach is not quantitative enough, and suffers from low resolution and observer bias. Therefore,

we are developing a more advanced method to characterize the 3D nuclear morphology of live neutrophils.

ER-Hoxb8 cells, immortalized murine hematopoietic progenitors, can be differentiated into mature neutrophils in vitro, recapitulating the stages of murine neutrophil differentiation. We have engineered Hoxb8 cells to constitutively express GFP in the nucleus with the LentiCRISPR-Cas 9 technology. Using the cutting-edge ZEISS Lattice Lightsheet7 microscope, we have optimized the quantitative analysis of changes in neutrophil nuclear morphology during their maturation live and in 3D. Moreover, we are currently developing a machine-learning algorithm to automatically segment and classify the stages of neutrophil differentiation.

Our results show that live 3D imaging is a valuable tool to study neutrophil nuclear morphology, as we can uncover features hidden in 2D images. This technology was used to study the changes in nuclear morphology in maturation-deficient cell lines. Moreover, we imaged differentiating cells during a 24h time-lapse experiment, allowing us to track changes in nuclear morphology over time in the same cells. This novel approach will assist in furthering fundamental neutrophil biology.

P.65 **The effect of co-signalling on the biogenesis and release of supramolecular attack particles by CD8+ induced T regulatory cells**

Margaritaki O.[1,2], Compeer E.B.[1], Larson J.H.[3], Hippen, K.L.[3], Puissegur M.P.[4], Valitutti S.[4], Blazar, B.R.[3], Harkiolaki M.[2], Dustin M.L.[1]

[1]Kennedy Institute of Rheumatology, University of Oxford, UK; [2]B24 Beamline, Diamond Light Source, UK; [3]Division of Blood and Marrow Transplantation, Department of Paediatrics, Masonic Cancer Centre, and The Centre for Immunology, University of Minnesota Medical School, Minneapolis, Minnesota, USA; [4]Centre de Recherches en Cancérologie de Toulouse, Institut national de la santé et de la recherche médicale, CEDEX 1, France

Cytotoxic T-cells (CTLs), regulatory T cells (Tregs) and natural killer (NK) cells each have the capacity to kill other cells in the context of immune response and homeostasis. They exert their cytotoxic function, in part, through the selective release of cytotoxic molecules such as granzyme B (GzmB) and Perforin 1 (Prf1). These molecules are delivered by lytic granules (LGs) into the synaptic cleft between the cytotoxic and target cell. Until recently, the consensus was that GzmB and Prf1 are released in soluble forms. However, our group used both cryo-Soft-X-ray Tomography (cryoSXT) and stochastic optical reconstruction microscopy, to reveal that these molecules are additionally released in non-vesicular extracellular particles of 111±36 nm, which we defined as Supramolecular Attack Particles (SMAPs). SMAPs consist of a glycoprotein shell, which can be detected by binding of the lectin wheat germ agglutinin (WGA), and a cytotoxic core including GzmB. CD2 co-stimulation is known to increase CTL and Treg function. Here, we have used correlative cryoSXT and 3D cryo-Structured Illumination Microscopy (cryoSIM) in a synchrotron setting, to study the morphological characteristics of LGs and SMAP presence in an NK cell line and CD8+ induced Tregs. Besides detecting instances of SMAP-like structures both released and within their LGs, we also investigated the effect of CD58 in supported lipid bilayers to demonstrate that CD2 co-signalling increases the release of SMAP-like particles by CD8+ iTregs, as indicated by increased co-localisation of GzmB and WGA using total internal reflection microscopy.

P.66 **Tonsil organoids reveal that plasmacytoid dendritic cells modulate the humoral immune response to ChAdOx1 nCoV-19**

Maria Fransiska Pudjohartono[1], Kate Powell[2], Helen Ferry[1], Ellie Barnes[1, 2, 3], Paul Klenerman[1, 2, 3*], Nicholas Provine[1, 4*]

[*]co-senior

[1]Translational Gastroenterology Unit, University of Oxford, UK; [2]Peter Medawar Building for Pathogen Research, Nuffield Department of Clinical Medicine, University of Oxford, UK; [3]NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK; [4]Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, UK

To understand how vaccines induce productive immune responses, it is critical to study the processes that occur at the site of immune priming -- secondary lymphoid tissues. However, access to such tissue in humans is limited. To overcome this, we have utilised a recently developed human tonsil organoid model to investigate the processes that regulate the induction of B cell and antibody responses to the ChAdOx1 nCoV-19 vaccine.

Innate responses were measured from activation-induced markers for all immune cells at 24 hours, adaptive responses were measured for B cells at 7 and 14 days after stimulation. Cytokine and antibody production were measured from culture supernatants. ChAdOx1 nCoV-19 induced early activation of all immune cells as well as B cell activation at later timepoints, with antigen-specific antibody production mirroring B cell activation.

Plasmacytoid dendritic cells (pDCs) showed the highest rate of transduction and activation, with pDC-depletion decreasing both innate and adaptive responses. IFN-α blockade produced similar effects, suggesting the role of pDC-derived IFN-α in the response to ChAdOx1 nCoV-19.

Studies report that type 1 IFN-secreting pDCs can mature into an antigen-presenting cell phenotype through a TNF-mediated process. This maturation process appeared to decrease pDC stimulatory capacity in this system, as blockade of TNF enhanced IFN-α levels and B cell activation.

Overall, this model reveals a critical role for pDCs through IFN-α production in the induction of B cell and antibody responses to the ChAdOx1 nCoV-19 vaccine. This model has potential for the study of immune responses to other vaccine platforms.

P.67 **The role of adaptive nk cells during acute hiv-1 infection with different subtypes**

Aljawharah Alrubayyi[1,7], Amin S. Hassan[2,3], Jonathan Hare[4,5], Jill Gilmour[6], Joakim Esbjörnsson[1,3], Eduard J. Sanders[1,2], Sarah Rowland-Jones[1], Dimitra Peppa[7,8]

[1]Nuffield Dept of Clinical Medicine, University of Oxford, UK; [2]KEMRI/Wellcome Trust Research Programme, Kilifi, Kenya; [3]Department of Translational Medicine, Lund University, Sweden; [4]International AIDS Vaccine Initiative (IAVI), Human Immunology Laboratory, Imperial College, London, UK; [5]International AIDS Vaccine Initiative (IAVI), New York, NY, USA; [6]Department of Infectious Disease, Imperial College, London, UK; [7]Division of Infection and Immunity, University College London, London, UK; [8]Mortimer Market Centre, Department of HIV, CNWL NHS Trust, London, UK

Background: Understanding the early immune response to HIV-1 infection represents a unique opportunity for the identification of novel targets for prophylactic/therapeutic approaches. Natural Killer (NK) cells are an important component of innate immunity that can modulate the pathogenesis of acute HIV-1 infection (AHI). However, the role of NK cells in mediating early host defence against infection with different HIV-1 subtypes, and clinical outcomes, remains poorly understood. Here, we studied the early imprinting effects of different HIV-1 subtypes and pro-inflammatory environment on the NK cell compartment in a unique cohort with AHI.

Methods: Participants with AHI were sampled longitudinally in four different sub-Saharan African sites under “IAVI protocol C” (n=25 subtype A, n=17 subtype C, n=7 subtype D). The median estimated days post-infection for subtype A, and non-subtype A was 32 and 35 days, respectively

(visit 1), and 95 and 92 days (visit 2). Multiparameter flow cytometry was used for phenotypic characterisation. NK cell ADCC responses were determined against antibody-coated Raji cells. The metabolic profile was assessed by a Seahorse technology. Plasma soluble markers were measured using multiplexed assays.

Results: NK cell subsets with adaptive/memory features expand during AHI with subtype A compared to non-A (visit 1 p=0.008, visit 2 p=0.005). This adaptive NK cell signature was delineated by lower expression of the transcription factor PLZF and was further enriched by higher expression of the activating receptor NKG2C and lower expression of the signalling molecule FcεRγ. Individuals with a high frequency of adaptive NK cells exhibited higher levels of IL-12p70 (p=0.03). Increased frequencies of adaptive NK cells were associated with lower HIV viral load (p=0.017) and higher CD4 T cell counts (>500). These phenotypic attributes were accompanied by enhanced NK cell ADCC capacity and higher IFN-γ (p=002) and TNF-α (p=0.0165) production in subtype A versus non-A. Notably, NK cell IFN-γ production correlated inversely with HIV-1 viral load (r=-0.343, p=0.03). The enhanced functionality of NK cells was reflected in their superior capacity for oxidative phosphorylation (p=0.035).

Conclusion: These data suggest that specific NK cell subsets could confer better HIV-1 control, highlighting their potential role as a prognostic marker and as a new target for the development of novel immunotherapeutic and 'cure' strategies.

P.69 **Improving nivolumab blocking efficiency through antibody engineering**

Martin Fellermeyer[1], Edward Jenkins[1], Judith Leitner[2], Peter Steinberger[2], David Klenerman[3], Simon J. Davis[1]

[1]MRC Human Immunology Unit, John Radcliffe Hospital, University of Oxford, UK; [2]Division of Immune Receptors and T cell Activation, Institute of Immunology, Medical University of Vienna, Austria; [3]Department of Chemistry, University of Cambridge, UK

There is an increasing number of immune-checkpoint inhibitors being developed and approved for cancer immunotherapy. Unfortunately, there is a lack of understanding on how to design the most efficacious blocking antibodies. This is likely influenced by the general characteristics of the respective receptor superfamily, but also structural nuances of each specific protein. Here, we focused on the PD-1 blocking antibody nivolumab. First, we demonstrated that nivolumab as well as pembrolizumab biosimilars (hIgG4) surprisingly retained agonistic activity in a FcgammaR-dependent manner. Next, to investigate this effect in mouse tumour models, we created a ‘murinised’ form of nivolumab (nivolumab mIgG1). Again, despite blocking PD-L1, nivolumab can act like an agonist in certain settings. Using different strategies of antibody engineering to either increase the overall dimensions of nivolumab or relocate the FcgammaR binding site, we successfully reduced this agonistic activity. Furthermore, we could show that while Fc-engaging nivolumab remained in ‘close contacts’ created by T cells, extended forms as well as nivolumab with a mutation to abrogate FcgammaR interactions were excluded. Finally, using mice humanised for PD-1, we compared the efficacy of nivolumab with and without FcgammaR binding activity. In line with other studies using anti-mPD-1 antagonists, nivolumab harbouring mutations to reduce FcgammaR binding demonstrated increased anti-tumour activity. Importantly, we offer a novel mechanism of action for the observed effects and extend this finding to the clinically used drug nivolumab.

P.70 **TIGIT: friend or foe in cancer development?**

Ashwin K. Jainarayanan[1,2], Jesusa Capera Aragones[1], Nithishwer Mouroug Anand[3], Sakina Amin[3], Ranjeet Mahla[1], Rachael Bashford-Rogers[3], Enas Abu-Shah[1], Michael L. Dustin[1] and Shivan Sivakumar[1,4,5,*]

[1]Kennedy Institute of Rheumatology, University of Oxford, Oxford; [2]Interdisciplinary Bioscience Doctoral Training Program and Exeter College, University of Oxford, UK; [3]Department of Biochemistry, University of Oxford, UK; [4]Oncology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; [5]Department of Oncology, University of Oxford, UK;
[*]Correspondence: Shivan.Sivakumar@exeter.ox.ac.uk (Shivan Sivakumar)

T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) is an inhibitory regulator which is critical in cancer immunotherapy. The tumor tissues with the upregulated expression of TIGIT exhibit aberrant immune characteristics. TIGIT blockades in cold tumour therapy is currently being widely investigated due to its promising nature in cancer immunity. The dual role of TIGIT in functioning both as an inhibitory and co-stimulatory molecule on T cells is of great significance to explore and validate effective immune-related targets. High levels of TIGIT expression seen to be associated with poor prognosis across multiple cancers. In this study, we conduced single cell pan-cancer analysis of tumour infiltrating cells. TIGIT ligand expression in TME was seen to triggers activation of TIGIT in tumour infiltrating population, specifically CD8 and Treg cells. We also studied the immune synapse formation with TIGIT ligand on lipid bilayer, to see how it influences T cell activation across T cell subtypes. We can see distinct TIGIT expression in tumour infiltrating T cells reflecting their profiles. Our study furthers insights on the role of TIGIT and guides the development of new avenues for pan cancer treatment.