Oral presentations

A role for human splenic tissue macrophages during the earliest phases of invasive disease

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Tissue macrophages are the main mechanism in innate cellular immunity involved in removal of pathogens from the bloodstream but information on their functionality in the human spleen is scarce. We have shown in mouse models that invasive bacterial disease starts after rare events of within-macrophage replication of bacteria which then shed back into the blood causing invasive disease. Through two clinical trials, we have access to human spleens and liver segments which can be maintained viable by ex vivo organ perfusion. Infection with Streptococcus pneumoniae shows efficient clearance by the different splenic macrophages sub-types including CD68+CD163+CD169- red pulp macrophages, CD68+CD163-CD169+ perifollicular sheath macrophages and CD68+CD163-CD169- periarteriolar sheath macrophages. These first observations at the whole organ level indicate that in both upon human red pulp and the perifollicular sheath macrophages, rare cells are permissive to intracellular bacterial replication. Apoptosis, visualised with anti-cleaved-caspase 3 antibodies, is observed about eight times more frequently in CD169+ macrophages when compared to other cell types. As in mice, the CD169+ macrophages appear to be “stressed” during encounter with invasive bacteria and we hypothesis that, also in humans, the CD169+ macrophages could be the weak element in our defence from invasive infection.

Vancomycin disrupts macrophage antifungal immunity

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Prolonged antibiotic use is a controllable risk factor for systemic candidiasis. In recent years, antibiotics have been shown to have off-target detrimental effects on immune cells, but whether antibiotics disrupt antifungal immunity is unknown. We recently found that the antibiotic vancomycin enhanced mortality from invasive candidiasis in mice, whereas other antibiotics (e.g. metronidazole) did not affect susceptibility. To examine the mechanisms by which vancomycin impairs anti-Candida immunity, we explored how vancomycin pre-treatment of bone-marrow derived macrophages (BMDMs) impacted their responses to C. albicans challenge. Vancomycin-treated macrophages had reduced fungal killing, although phagocytosis of yeast cells was only modestly affected. Instead, vancomycin-treated macrophages exhibited impaired mitochondrial function, in which they failed to upregulate their respiratory capacity when stimulated with Candida. We used confocal microscopy to examine the mitochondria, which showed reduced mitochondrial mass, reduced membrane potential and morphological changes in vancomycin-treated macrophages that have been previously linked to dysfunctional metabolic activity. Indeed, both an unbiased metabolite screen and bulk RNA-sequencing revealed several dysregulated pathways linked with mitochondrial dysfunction. For example, vancomycin-treated macrophages have an early upregulation of genes involved in inflammasome activation and pyroptosis, along with enhanced expression of mitochondrial-localised anti-inflammatory enzymes and lipids that have not been previously explored in the context of antifungal immunity. Our ongoing studies aim to determine which of these mediators prevents fungal killing by the vancomycin-treated macrophages. Taken together, our results improve our understanding of the pathways regulating antifungal immunity in macrophages and suggest that antibiotic-induced susceptibility to C. albicans may be partly driven by disrupted macrophage function.

Complement and inflammatory marker analysis in women with obstetric antiphospholipid syndrome (OAPS)

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Background
Recurrent pregnancy loss (RPL) affects >6000 couples/year in the UK, having significant physical and psychological consequences. Antiphospholipid syndrome (APS) is an autoimmune condition, associated with increased thrombosis risk. APS is commonly identified through its obstetric features (OAPS), being the most frequent treatable RPL risk factor. However, the mechanism underlying RPL-APS association remains unidentified.
Methods
C3a, C5a, SC5b-9, annexin V, cytokine, and cytolytic molecule concentration in the plasma and serum of women with OAPS (N=44), RPL without APS (RPL) (N=48), and healthy controls (N=50) were investigated via ELISA.
Results
Both C3a (p=0.0052) and IL-5 (p=0.0044) levels were elevated in OAPS, compared to RPL. Annexin V was reduced in both the RPL and OAPS cohorts (p=0.0069), while Granzyme B appeared decreased in RPL (p=0.0266), but not OAPS.
Conclusions
Increased C3a in OAPS indicates complement dysregulation, although not affecting the terminal complement complex (SC5b-9). Elevated IL-5, also seen in OAPS, may promote B cell survival and differentiation, enhancing autoantibody production.
Reduction in annexin V, which inhibits prothrombin activation, was not limited to OAPS, but also seen in the RPL cohort. Interestingly, granzyme B decrease, which is also linked to the coagulation/fibrinolysis pathway dysregulation, was only seen in women with RPL without APS. Although RPL has not been independently associated with thrombosis, our findings indicate that pro-thrombotic dysregulation underlies this condition, regardless of APS status.

We are aiming combine this work with antiphospholipid antibody profile assessment, to identify women in increased risk of subsequent pregnancy loss, promoting a personalised approach to antenatal treatment.

Large E-cadherin+ CD103+ CD8+ T cells invade biliary epithelial cells through interactions with β-catenin

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Background
The liver is a hotspot for lymphocyte-based cell-in-cell structures (CICs). Recently, the presence of CD8+ T cells within biliary epithelial cells (BEC) has also been demonstrated histologically. We aim to further characterise these T cells and their mechanism of internalisation.
Methods
We developed a high-content imaging (HCI) co-culture platform to quantify and characterize the internalisation of human T cells into primary human BEC. This assay was used to observe the effect of T cell receptor (TCR) activation, to compare CD8+ T cells to CD4+ T cells, and to investigate the effect of small molecular inhibitors. T cell phenotyping was performed in vitro using flow cytometry and in vivo using immunohistochemistry (IHC). Individual sub-populations of CD8+ T cells were isolated using fluorescence-activated cell sorting (FACS).
Results
CD8+ T cell internalization into BEC was more frequent amongst larger cells and 48 hr following TCR-mediated activation. CD8+ T cells invaded BEC more frequently compared to CD4+ T cells. CD8+ T cells expressed CD103+ and CD69+ both in vitro and in vivo when internalising into BEC. Inhibitors of Phosphoinositide 3-kinase (PI3K) signaling, and actin remodeling, significantly reduced this invasion. E-cadherin expression by CD8+ T cells correlated with frequency of internalisation. Furthermore, CD8+ T cells interactions with BEC surfaces were enriched with E-cadherin and β-catenin. FACS-sorted E-cadherin+ CD8+ T cells were larger than E-cadherin- CD8+ T cells and invaded BEC more frequently.
Conclusions
We unveil a distinct cell-in-cell structure in the liver, whereby BEC are invaded by E-cadherin+ CD103+ CD69+ CD8+ T cells.

Blockade of lag3 synergizes responses to anti-PD-L1 through increasing duration of T cell receptor signalling

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Background: PD-1 and its ligand PD-L1 are attractive candidates for cancer immunotherapy and their blockade has shown clinical efficacy in several cancer types. We recently developed a new metric, “TCR.strong”, that represents five genes (IRF8, OX40, STAT4, TNIP3, ICOS) that are specifically upregulated in response to anti-PD1 immunotherapy in CD4+T cells. However, despite these successes, most patients fail to respond to single agent immunotherapy and adverse event rates are frequently high. Combinations of immunotherapy have largely been selected on an empirical basis and deeper mechanistic insight is lacking. Here we sought to rationally identify more effective immune checkpoint blockade (ICB) combinations.

Methods: We utilised an accelerated adaptive tolerance model, using Tg4 Nr4a3-Tocky mice to explore T cell phenotypic changes in response to anti-PD-L1 or combinatorial anti-PD-L1 and anti-Lag3 immunotherapy. We performed RNAseq on rechallenged cells and modulated the length of TCR sinalling using pharmalogical inhibitor to understand how TCR sinal duration regulates key T cell activation markers.

Results: Combination therapy increased T cell activation and prolonged expression of the TCR.strong metric, which was driven by an increased duration of TCR signalling. RNAseq revealed that combinatorial therapy sustained a wide range of genes indicative of strong TCR signalling, which was not the case for monotherapy. In addition, T cells showed enhanced metabolic enzyme expression and ribosomal biogenesis.

Conclusions: Our data show that co-blockade of PD-L1 and Lag3 results in synergistic T cell activation which is driven by an increased duration of TCR signalling, which results in augmented metabolism and biosynthesis.

Phosphoinositide acyl chain saturation drives CD8+ effector T cell signaling and function

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How lipidome changes support CD8+ effector T (Teff) cell differentiation is not well understood. Here we show that, although naive T cells are rich in polyunsaturated phosphoinositides (PIPn with 3–4 double bonds), Teff cells have unique PIPn marked by saturated fatty acyl chains (0–2 double bonds). PIPn are precursors for second messengers. Polyunsaturated phosphatidylinositol bisphosphate (PIP2) exclusively supported signaling immediately upon T cell antigen receptor activation. In late Teff cells, activity of phospholipase C-γ1, the enzyme that cleaves PIP2 into downstream mediators, waned, and saturated PIPn became essential for sustained signaling. Saturated PIP was more rapidly converted to PIP2 with subsequent recruitment of phospholipase C-γ1, and loss of saturated PIPn impaired Teff cell fitness and function, even in cells with abundant polyunsaturated PIPn. Glucose was the substrate for de novo PIPn synthesis, and was rapidly utilized for saturated PIP2 generation. Thus, separate PIPn pools with distinct acyl chain compositions and metabolic dependencies drive important signaling events to initiate and then sustain effector function during CD8+ T cell differentiation.

Poster presentations

P.01 **Using fluorescent ligands as flow cytometry probes: a study of adenosine receptor subtypes expression in human macrophages**

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Adenosine is a molecule responsible for tissue homeostasis, with upregulation of levels in stressed/damaged tissues. In the tumour microenvironment, high levels of adenosine lead to suppression of immune cells which are attempting to attack or control the tumour, resulting in failure of immunotherapy. Adenosine signals via four G-protein coupled receptors: A1, A2A, A2B and A3. These have been shown to be expressed at the gene level in macrophages, one of the key immune cells in the tumour.

Because of their complex structure, it is difficult to generate traditional antibodies against the adenosine receptors (AR), making flow cytometry analysis of AR expression challenging. Recently, we have been developing fluorescent ligands based on small molecules specific to AR as a tool for characterising AR expression and function on primary cells. Two ligands specific to the A2A and A2B ARs have been generated and validated and are highly selective antagonists of the respective AR. We used these newly synthesised fluorescent ligands to investigate the expression profile of A2A and A2B AR in human primary macrophages via flow cytometry. Preliminary data indicate that these ligands bind and can be displaced by unlabelled ligand, indicating AR-specific binding on macrophages, and once confirmed, will demonstrate the utility of these ligands to characterise AR levels on primary cells. Depending on macrophage subset, results indicate different levels of A2A and A2B. Future work aims to study the role of macrophage polarisation on AR profile and levels, to confirm which AR should be primary therapeutic targets in cancer immunotherapy.

P.02 **Streptococcus uberis exploits NLRP3 inflammasome activation in disease pathogenesis: the role of the cell envelope serine protease SUB1154**

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Bovine mastitis is an intramammary inflammatory disease which severely impacts dairy productivity, animal welfare and the sustainability of milk production. Streptococcus uberis is the major cause of bovine mastitis in the UK. In contrast to other mastitis pathogens, S. uberis does not stimulate an innate response from epithelial tissues; initial recognition of infection is via macrophages found within milk. Paradoxically, triggering this defence response promotes bacterial colonisation.

Challenge studies with S. uberis in dairy cattle have indicated the importance of a bacterial cell surface serine protease (SUB1154). This protein is essential for high level colonisation. Initial investigation indicated that this protein activated the NLRP3 inflammasome in bovine mammary macrophages resulting in the release of IL-1β (cytokine indicative of NLRP3 activation). The aim of this project is to determine the role of the SUB1154 protein in the inflammasomal pathway.

Bovine mammary macrophages isolated from milk were challenged with heat-killed strains of S. uberis and/or purified SUB1154. In the absence of SUB1154, IL-1β is not produced, but production of IL-1β was restored following supplementation with the purified protein, indicating the importance of SUB1154 in the NLRP3 inflammasomal pathway. Current analysis suggests that SUB1154 primes the NLRP3 inflammasome intracellularly via TLR2.

P.03 **The impact of ruxolitinib and tocilizumab on IL-6 evoked phospho-STAT3 induction across human immune cell subsets**

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Designing suitable biomarker assays to evaluate target engagement of therapeutic agents supports hypothesis testing in clinical trials. As an example, we have used phospho flow cytometry to investigate the phosphorylation of STAT3 in response to the inflammatory cytokine IL-6 across various human immune cell subsets including CD4 and CD8 T cells, NK and NK-T cells, Tregs, B cells and monocytes. IL-6 differentially induced phospho-STAT3 across immune subsets.

We have used this assay to compare two therapeutic agents targeting the IL-6 pathway, tocilizumab and ruxolitinib. Tocilizumab blocks IL-6 signaling at the IL-6 receptor whereas ruxolitinib inhibits IL-6-evoked STAT3 phosphorylation through inhibition of JAK1/2. We compare and contrast the sensitivity of human immune cell subsets to these two inhibitors by measuring the induction of STAT3 phosphorylation by a 'low' and 'high' concentration of IL-6.

On-going studies are utilising this approach to investigate the nature of IL-6 signaling in health and disease.

P.04 **Effect of GM-CSF on the immune response of human monocytes against C. albicans**

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine known for its role in regulating inflammation during fungal infections. GM-CSF can promote survival, adhesion and movement of immune cells such as monocytes, macrophages, and neutrophils and increases antifungal activity during infections, by promoting production of reactive oxygen species, phagocytosis, and cytokine secretion. This research aims to study the interaction between human inflammatory monocytes and C. albicans, focusing on the role of C-type lectin receptors (CLRs). CLRs have been implicated in fungal recognition by binding components of the fungal cell wall. We hypothesize that by investigating GM-CSF-driven changes in monocytes during C. albicans infection, we will unveil novel biological processes implicated in protection against infection. These findings will inform on the suitability of GM-CSF as a therapeutic agent against fungi. We have established an infection assay in which GM-CSF-treated monocytes are exposed to C. albicans using monocytes treated with macrophage colony-stimulating factor (M-CSF) as controls. GM-CSF-monocytes upregulate the activation markers CD11b and HLA-DR, and CLRs CD206, Dectin-1 and CLEC5A compared to M-CSF-monocytes. They also display enhanced phagocytic activity against zymosan. We expose GM-CSF and M-CSF-monocytes to C. albicans (strain CS-5314) (MOI=1, 37oC, in the presence of human serum) for 1 and 3 h. These conditions promote hyphae formation hence monocytes are exposed to C. albicans displaying a major virulence attribute. Read outs include CFUs, cytokine production and microscopic examination. CFU were reduced dramatically during the assay at 1 and 3 h in the presence and absence of monocytes with a slight increased when monocytes (GM-CSF or M-CSF-treated) are present. GM-CSF promotes secretion of TNF-α, IL-6 and IL-1β in response to infection with some TNF-α already produced by uninfected GM-CSF-monocytes. Preliminary image analysis confirmed rapid hyphae formation during infection to generate a long hypha after 3 h in the absence of monocytes. Importantly, presence of monocytes reduced hyphae formation, with this effect being more evident in the case of GM-CSF-treated cells. Further work aims to further characterise the impact of GM-CSF on monocyte responses to C. albicans infection and the contribution of specific signalling pathways to these responses.

P.05 **A family-wide assessment of latent STAT transcription factor interactions reveals divergent dimer repertoires**

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The conversion of STAT proteins from latent to active transcription factors is central to cytokine signalling. Triggered by their signal-induced tyrosine phosphorylation, it is the assembly of a range of cytokine-specific STAT homo- and heterodimers that marks a key step in the transition of hitherto latent proteins to transcription activators. In contrast, the constitutive self-assembly of latent STATs and how it relates to the functioning of activated STATs, is understood less well. To provide a more complete picture, we developed a co-localization-based assay and tested all 28 possible combinations of the seven unphosphorylated STAT (U-STAT) proteins in living cells. We identified five U-STAT homodimers —STAT1, STAT3, STAT4, STAT5A and STAT5B— and two heterodimers —STAT1:STAT2 and STAT5A:STAT5B— and performed semi-quantitative assessments of the forces and characterizations of binding interfaces that support them. One STAT protein —STAT6— was found to be monomeric. This comprehensive analysis of latent STAT self-assembly lays bare considerable structural and functional diversity in the ways that link STAT dimerization before and after activation.

P.06 **Exploring Affinity-Dependent Metabolic Imprinting of Anti-Viral T Cell Responses**

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T cell responses to viral infections are mediated by their recognition of specific viral peptide-MHC complexes by the T cell receptor (TCR). T cell functions are underpinned by changes in metabolism in response to TCR ligation, whereby the metabolic network is reprogrammed to meet the demands of the cell and support cell survival and growth. This project is focused on Epstein-Barr virus (EBV) antigen-specific T cells; primary EBV infection affects over 90% of the worldwide population and in some individuals, long-term carriage of EBV infection is associated with the development of Multiple Sclerosis (MS), numerous cancer types (eg. endemic Burkitt's lymphoma) and lymphoproliferative diseases.

To investigate the immune profiles and metabolism of EBV antigen-specific T cells, preliminary experiments have assessed the HLA type of healthy donors and detected EBV antigen-specific T cells using peptide-MHC tetramers. Tetramer staining has been combined with metabolic probe staining, to assess mitochondrial mass and membrane potential, as well as fatty acid uptake. Tetramer staining has also been combined with SCENITH analysis, to explore the dependence of cells on certain metabolic pathways.

This project aims to explore the relationship between TCR binding affinity and the phenotype of EBV-specific T cells. Therefore, going forward, peptide-specific T cells will be expanded in vitro, to generate T cell clones with unique TCRs and of different affinities for further metabolic exploration.

P.07 **Hypoxia impairs CD8+ T cell effector function and metabolic reprogramming**

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Introduction
Hypoxia in the tumour microenvironment (TME) may drive immunosuppression and progression of disease. We describe CD8+ T cell anti-tumour function in hypoxia by analysing immune functions, metabolism, and signalling pathways, and interrogating underlying mechanisms.
Methods
CD8+ T cells isolated from human peripheral blood are incubated overnight in normoxia (21% oxygen) or hypoxia (1% oxygen) to allow oxygen level equilibration. Subsequently, cells are activated

by anti-CD3/anti-CD28 antibodies and analysed.
Results
CD8+ T cells stimulated under hypoxia demonstrate impaired T cell activation (CD25 expression) and profound suppression of IFN-□ release, but not TNF-□. Granzyme-B expression, CD107a externalisation, and tumour cell killing are also unaffected by hypoxia indicating distinct effects on specific T cell functions. A defect in CD28, but not T cell receptor signalling, is observed in hypoxia, accompanied by reduced NFAT translocation to the nucleus. This signalling defect lies at the level of mTOR with impact on downstream targets. Analysis of distinct CD8+ T cell subpopulations confirmed hypoxia impacts all to a similar extent, including memory populations that encounter antigen in the hypoxic TME. Stable isotope-based metabolic tracing identified that activation-induced increases in glycolysis, glucose oxidation, and glutaminolysis are reduced in hypoxic vs. normoxic cells – consistent with impaired mTOR activity – which may underpin their impaired effector function.
Conclusion
Hypoxia impairs CD8+ T cell signalling, activation and specific effector functions. This occurs alongside diminished metabolic reprogramming upon activation. Further work will interrogate the mechanistic basis of mTOR suppression and interrogate activity of these pathways within patient tumour samples from hypoxic TMEs.

P.08 **Impaired macrophage phagocytosis of non-typable Haemophilus influenzae in older adults is associated with reduced CD36 expression**

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Advanced age is a risk factor for pulmonary infections, which are the leading cause of death in older adults. The mechanism behind increased susceptibility to infection is poorly understood, but changes in macrophage function, including phagocytosis, due to inflammageing may be implicated.

Monocyte derived macrophages (MDM) from healthy young adults (<35 years) and non-frail older adults (>65 years, frailty score 1-3) were exposed to heat-killed, fluorescently labelled Non-typable haemophilus influzenzae (NTHI), Streptococcus pneumoniae (SP) or apoptotic neutrophils (AN), and phagocytosis measured by flow cytometry, alongside co-current expression of scavenger receptors CD36, TLR2 and TLR4.

MDM phagocytosis of NTHI was significantly reduced in older adults compared to young (61% vs. 33%, $p<0.05$). Phagocytosis of SP, and efferocytosis of AN was not changed. Baseline CD36 expression was significantly reduced in older vs. young adults (60% vs. 29%, $p<0.05$). In older adults, MDM phagocytosis of NTHI increased CD36 expression compared to baseline (47% vs. 29%, $p<0.05$) but remained significantly decreased compared to young adults (47% vs. 70%, $p<0.05$), whereas phagocytosis of SP ($p<0.05$) and efferocytosis of AN ($p<0.01$) increased CD36 expression to that of younger adults. There was no change in expression of TLR2 or TLR4.

Impaired phagocytosis of NTHI by older adult macrophages is associated with impaired ability to elevate CD36 expression. CD36 forms heterodimers with TLR2 and TLR4 to aid LPS recognition, and trigger signalling cascades. Thus, impaired CD36 regulation presents a mechanism for dysregulated NHTI phagocytosis in older adults, which may be targeted as a novel therapy for age-related infections.

P.09 **Therapeutic potential of a multi-tumour antigen peptide vaccine for hard-to-treat cancers**

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Glioblastoma (GBM), advanced prostate cancer (PCa) and triple negative breast cancer (TNBC) have poor survival rates and limited treatments. Targeting residual tumour cells following treatment can prove crucial to avoid recurrence, with vaccines representing an appealing strategy due to their potential to engage the immune system. Cancer/testis antigens HAGE and NY-ESO-1 as well as tumour antigen WT1 are promising candidates for vaccine development; their expression can be

upregulated by low dose decitabine (DAC) treatment, a DNA methyltransferase inhibitor. In addition, the novel adjuvant CAF®09b can induce CD8+ T cell responses against these antigens. A panel of human GBM, PCa and TNBC cell lines was treated with DAC and tested for these antigens using qPCR and western blot. TNBC tissue microarrays were probed for the 3 antigens using immunohistochemistry (IHC). Peptide sequences derived from these antigens were combined with CAF®09b and used for the immunisation of HHDII/DR1 mice. Antigen-derived peptides were tested for immunogenicity using an IFNg ELISpot assay and antigenic activation of T cells from blood was assessed by flow cytometry using pentamer staining. The expression of the 3 antigens in GBM, PCa and TNBC cell lines indicated that low-dose DAC treatment induced antigen upregulation at the transcript and protein levels. IHC staining of human TNBC confirmed the expression of the 3 antigens. The splenocytes derived from CAF®09b-adjuvanted HAGE/NY-ESO-1/WT1 vaccine-immunised mice showed strong IFNg responses, and the pentamer-stained blood suggested the presence of antigen-activated CD8+ T cells. Our vaccine is therefore a good candidate for vaccine development with clinical potential.

P.10 **Therapeutic efficacy of a prostatic acid phosphatase (PAP) derived peptide-based vaccine administered with CAF®09b adjuvant tested in HHDII/DR1 and C57BL/6 mice**

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Sipuleucel-T, the only available FDA approved vaccine for advanced prostate cancer has extended the overall survival of the patients by 4.1 months, but it remains expensive and only available in the US. Dr. McArdle et al group has identified and patented a mutated PAP-derived 42 (MutPAP42mer) amino-acid long sequence. The immunological properties of this PAP derived 42mer peptide together with a 15 AA long PAP derived sequence (PAP15mer) was investigated in two mouse models, HHDII/DR1 and C57BL/6 mice with the use of novel cationic liposomal adjuvant CAF®09b. Vaccine specific immune responses were measured by ELISPOT assay. CD3+ T cells were isolated from splenocytes, stimulated ex-vivo with IL2 and PAP peptides and were co-cultured with PCa cell lines to assess cytotoxic capability by using 'CCK-8 Cell counting kit'. Results obtained so far confirm the immunogenicity and in-vitro cytotoxic efficacy of Mut PAP42mer vaccine using CAF®09 as an adjuvant. Mut 42mer vaccine could induce CD8+ and CD4+ specific responses and the vaccine specific T cells were able to recognise and kill PCa target cell lines in-vitro. Presence of PCa associated MDSCs were also identified. Immunosuppressive nature of TME associated with PCa, makes immunotherapies less effective in treating PCa. MutPAP42mer/CAF®09 is both immunogenic and able to generate T-cells capable of recognising and killing PAP-expressing targets. We will also assess wether blocking B2 adrenergic receptor mediated signalling along with PAP-derived 42mer peptide with CAF®O9 vaccine impact the growth of TRAMP-C2 cells injected into C57BL/6 mice.

P.11 **Engineering amino acid uptake promotes CAR T-cell adaption to the tumour environment**

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Cancer cells take up amino acids from the extracellular space to drive cell proliferation and viability. Similar mechanisms are applied by immune cells, resulting in the competition between conventional T cells, or indeed chimeric antigen receptor (CAR) T cells and tumour cells, for the limited availability of amino acids within the environment. We demonstrate that T cells can be re-engineered to express SLC7A5 or SLC7A11 transmembrane amino acid transporters alongside CARs.

Transporter modifications increase CAR T-cell proliferation under low tryptophan or cystine conditions with no loss of CAR cytotoxicity or increased exhaustion. The adaptation of CAR-T to tumour environment, results in an increase of the AML clearance in murine models.

Transcriptomic and phenotypic analysis reveals that downstream, SLC7A5/SLC7A11–modified CAR T cells upregulate intracellular arginase expression and activity. Thus, CAR T cells can be adapted to the amino acid metabolic microenvironment of cancer, a hitherto recognized but unaddressed barrier for successful CAR T-cell therapy.

P.12 **Re-wiring of TCR signalling pathways during CD8 T cell exhaustion**

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Expression of Nr4a family genes is a biomarker of TCR engagement, and fluorescent reporters of Nr4a gene expression can be used to track TCR signals. Comparative analysis of Nr4a1 and Nr4a3 reporter systems has revealed differential regulation by signalling pathways that diverge downstream of the TCR. Nr4a1 expression, which is independent of NFAT signalling, is responsive to low strength signals such as positive selection in the thymus and tonic self-antigen recognition in the spleen. In contrast, NFAT dependent Nr4a3 expression requires stronger TCR signals.

We utilised sub-cutaneous injection of MC38 carcinoma cells to model chronic antigen recognition in the tumour microenvironment. Whilst antigen specific CD8 TILs do express Nr4a1 during early stages of tumour development, it is shut down by day 13. This is associated with a concurrent up-regulation of negative regulators of TCR signalling such as PD-1 and CD39. Nr4a1 expression was restricted in both SLAMF6- TIM-3+ exhausted CD8 T cells and SLAMF6+ TIM-3- stem-like CD8 T cells. Nr4a1 expression was unaltered by blockade of PD-L1 and injection of anti-CD3 only drove Nr4a1 expression in a small subset of cells.

In contrast to Nr4a1, Nr4a3 expression is maintained in CD8 TILs. This suggests that chronically stimulated CD8 T cells can sustain NFAT signalling whilst shutting down other pathways. In vitro chronic stimulation of purified CD8 T cells demonstrated typical features of exhaustion, and re-capitulated reduced Nr4a1 expression.

P.13 **Preventing graft-versus-host disease by redirecting TREG specificity with TCR retroviral transduction**

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Background: Foxp3+ regulatory T cells (Treg) have been shown to play a central role in immune tolerance and the suppression of transplant rejection. Redirecting the specificity of Treg to alloantigen may enhance their therapeutic potential, however, the persistent presence of alloantigen may lead to Treg exhaustion/deletion. Therefore, we propose that generating Treg that recognise exogenous molecules may limit the timeframe and site of activation which may limit the aforementioned caveats of this approach.

Methods: Murine splenic Treg were isolated and expanded with anti-CD3/CD28 beads in the presence IL-2 and TGFβ in vitro. A viral vector containing the rearranged Vα14 and Vβ7 chains of an iNKT T cell TCR was generated and used to retrovirally transduce expanded Treg on day 2; the transduction efficiency was determined by flow cytometry and the suppressive capacity of such Treg was evaluated using a mixed lymphocyte reaction (MLR) in vitro.

Results: We found that Treg cultured with beads and IL-2/TGFβ underwent significant expansion with limited contaminant cells. Additionally, expanded Treg were receptive to transduction as 60% expressed a functional iNKT TCR compared to mock transduced Treg after 7 days. Moreover, purified transduced Treg were able to suppress alloreactive T cell proliferation and expansion in the presence of αGalCer-CD1d.

Conclusion: Taken together, we have shown that Treg can be retrovirally-transduced with an iNKT TCR that re-directs specificity to glycolipid/CD1d and increases suppression ability. Therefore, this study suggests that redirecting the specificity of Treg to known antigens may enhance the ability of such cells to control allograft rejection, GVHD and autoimmunity.

P.14 **Mass Cytometry at The University of Birmingham Flow Cytometry Facility: Supporting Researchers' Needs across the Midlands and the UK**

Shahram Golbabapour, Ferdus Sheik, Guillaume Desanti, Adriana Flores-Langarica

MDS, Technology Hub, Flow Cytometry Facility, University of Birmingham, UK

The CyTOF mass cytometry technology allows high parameter single cell analysis that has been utilised in various research fields over the past two decades. The University of Birmingham Flow Cytometry Facility provides a CyTOF mass cytometry sample acquisition service in compliance with good clinical practice guidelines, making this instrument suitable for clinical trial projects. We provide user support in partnership with Standard Biotools to facilitate access since the experimental design to sample acquisition. We are just going to start working with Cytobank to facilitate some guidance on data analysis. Our CyTOF service is offered to any research partners from across the Midlands to the rest of the UK. In addition to the organisation of seminars and webinars, our on-site CyTOF expert is actively involved in the creation of educational and scientific events and material in partnership with MI TALENT participating in events such as the Festival of Learning and the WinterFest.

P.15 **The University of Birmingham Flow Cytometry Facility: Supporting Flow Cytometry Researchers' Needs across the Midlands and the UK**

Ferdus Sheik, Shahram Golbabapour, Guillaume Desanti, Adriana Flores-Langarica

MDS, Technology Hub, Flow Cytometry Facility, University of Birmingham, UK

Flow cytometry is a powerful technique that enables researchers to analyse and sort cells based on their physical and phenotypic properties. The University of Birmingham Flow Cytometry Facility plays a crucial role in supporting the research produced within and outside the University by providing access to state-of-the-art instruments, training and expertise to its users. Our equipment portfolio includes four Fortessa (4 to 5 lasers), two CytoFlex S (four lasers), one Attune NxT (2 lasers), one ImageStream MkII (6 channels), one Helios mass cytometer, one ID7000 spectral cytometer (5 lasers) and two FACSAria Fusion cell sorters (5 lasers). Our Helios and one of our Fortessa are compliant with good clinical practice guidelines, which makes them eligible for clinical trial projects. Moreover, our team works in collaboration with our users and Academic Leads to identify the next generation technologies that will support the competitivity and success of the future research conducted with our support. The University of Birmingham Flow Cytometry Facility is also a member of Midlands Innovation Flow Cytometry Group.

P.16 **Application of Tg4 Nur77-Tempo mice to identify effective immunotherapy combinations**

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Nur77, part of the Nr4a receptor family, reporter mice are valuable tools for studying T cell receptor (TCR) signalling. Nur77 is activated by TCR signalling and plays key roles in T cell differentiation. We have previously reported, using a GFP reporter system, that Nur77 is highly sensitive to TCR signalling, making it a key model to explore moderate T cell responses.

Utilising our recently developed Nur77-Timer rapidly expressed in lymphocytes (Tempo) reporter mice, we have further explored the dynamics of Nur77 expression following in vivo TCR stimulation of Tg4 Nur77-Tempo mice with primary doses of 4Y myelin basic protein peptide ([4Y]-MBP), followed by subsequent [4Y]-MBP rechallenge. Utilising flow cytometric analysis, we investigated Nur77-Tempo dynamics as well as expression of membrane receptors associated with strong TCR

signals (ICOS and OX40) in our system. We explore the effects of agonistic and inhibitory checkpoint modulation prior to peptide restimulation, both in vivo and in vitro.

Our data using Nur77-Tempo mice has revealed that anti-PD-L1 therapy leads to increased TCR signal strength during re-challenge. In addition, we create a novel in vivo/in vitro hybrid platform to screen for potential effective immunotherapy combinations that boost T cell activation.

We show, using the Tg4 Nur77-Tempo model, that Nur77-Tempo mice are a powerful tool for studying T cell responses to a wide range of TCR signal strengths. In addition, we show that Nur77 expression increases proportionally with the dose of peptide rechallenge received, with PD-L1 therapy augmenting T cell activation in vivo.

P.17 **Mapping the immune landscape: Optimising the multiplex immunohistochemistry images of Tissue-Resident Memory T cell in Breast Cancer**

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Background: Tissue-resident memory T cell (TRM) have been described as tissue resident non-circulating memory T cells. The TRM gene signature has been shown to be a favourable prognostic marker in triple-negative breast cancer (TNBC) in a study using single-cell sequencing . CD39 is upregulated on both CD4 and CD8 T-cells upon TCR stimulation. Futhermore, CD8+CD39+CD103+ has been shown to define activated tissue resident CD8 T-cells. The aim of this study was to optimise a panel of antibodies for multiplex fluorescence immunohistochemistry(mIHC) to study the composition and spatial location of TRM in tumours from 2000 breast cancer patients.

Method: A panel of 6 antibodies and DAPI were optimised for mIHC . CD103, CD39, CD8 and CD4 served as TRM markers, while pan-cytokeratin was used as a marker for tumour epithelium.

Results: Our mIHC optimising result showed the ideal condition for each marker. Phenotype subtypes can be divided into single-positive, double-positive, triple-positive, and negative-stained cells detected in both intra-epithelial and intra-stromal in breast cancer tissue microarray using Inform software (machine learning).

Conclusion: This research indicated an optimal condition of the mIHC stain for Nottingham breast cancer, which will be applied for staining a tissue microarray containing tumour cores from 2000 breast cancer patients.

P.18 **Blood Leukocyte Characterisation in Eosinophilic Granulomatosis with Polyangiitis (EGPA)**

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Rationale: Peripheral blood leukocyte characteristics are poorly understood in patients with Eosinophilic Granulomatosis with Polyangiitis (EGPA), a rare disease whereby blood and tissue eosinophilia feature.
Objectives: We wanted to measure the absolute counts of leukocytes in the blood of EGPA patients, specifically granulocytes (eosinophils, neutrophils, basophils), type 2-polarised cells (Type 2 Innate Lymphoid cells (ILC2s)) and T cell subsets (T1, 2, 17). We also wanted to asses eosinophil activation status at baseline.
Methods: Lithium-heparin whole blood was collected from stable EGPA patients (n=5) and healthy volunteers (n=9). Leukocyte populations were measured using cytometry. Absolute cell counts are presented per 100uL. Activation status was measured using CD11a/CD18, with results presented as a ratio to FMO control, with a low ratio meaning less activation. EGPA patients were on continued benralizumab, mepolizumab and/or oral steroid at time of blood collection.
Results: Granulocyte counts were similar between EGPA and health. ILC2 and B cell counts were

significantly lower in EGPA compared to health (p=0.027 and p=0.019 respectively). All T helper (Th) and cytotoxic T (Tc) cell subsets were similar, however Th17 cells counts were significantly lower in EGPA than in health (p=0.017). Baseline eosinophil activation in healthy donors was bimodally distributed. Eosinophil activation in EGPA was similar to lowly activated eosinophils in health (p=0.083).
Conclusion: ILC2, B and Th17 cells are decreased in EGPA patients compared to health. There is heterogeneity present in eosinophil activation between healthy donors, which warrants further investigation to establish biological and clinical relevance.

P.19 **Defining the surface proteome of apoptotic cell-derived extracellular vesicles**

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As cells die in vivo they are removed by phagocytes in a process that resolves inflammation and prevents disease. For clearance of dying (apoptotic) cells to be efficient and timely, dying cells release extracellular vesicles (EV) to attract phagocytes to sites of cell death. Crucially, this interaction of EV with the immune system underpins the control of inflammation, a process central to health, regenerative medicine and many inflammatory diseases.
Multiparametric single-EV analysis is a next generation approach enabling in-depth characterisation and identification of novel EV subpopulations. A significant challenge, however, is EV heterogeneity. In particular, EV vary greatly in size (<50nm - >1μm). NanoFCM have developed a novel platform that combines the capabilities of flow cytometry at the nano-scale with high sensitivity (40nm limit of scatter detection) and specificity (simultaneous scatter and fluorescence detection). This will enable comprehensive EV phenotyping at a scale that has previously been difficult to detect. Through collaboration between Aston University and NanoFCM, the EV surface will be probed at high resolution to establish a fundamental atlas of the surface proteome of apoptotic cell-derived EVs (ACdEVs) across the entire size range.
ACdEV surface proteome will be linked to function as we address the following key questions: what are the key components of ACdEV that enable communication with the immune system? Do ACdEV of differing sizes and sources interact in a similar manner with the immune system? Is it possible to produce a synthetic ACdEV with a defined surface proteome to mimic of ACdEV function?

P.20 **Increased IEL expansion following Salmonella infection in NAIP-deficient mice**

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Maintenance of intestinal integrity is dependent on crosstalk between epithelial, stromal and immune cells and the gut microbiota. Nod-like receptor (NLR) apoptosis inhibitory proteins (NAIPs) activate the NLRC4 inflammasome upon recognition of gram-negative bacteria, leading to pyroptosis/apoptosis, intestinal epithelial cell expulsion and release of IL-1β, IL-18 and prostaglandin E2 (PGE2). This is essential to limit bacterial dissemination, with NAIP KO mice experiencing gut epithelial barrier collapse four days after Salmonella infection, driven by TNF release. NAIPs also appear to have homeostatic roles within the intestinal epithelium, as our group has previously shown that NAIPs suppress colonic tumourigenesis but enhance colonic inflammation. We aimed to further understand the role of NAIPs on the intraepithelial lymphocyte compartment during infection with Salmonella Typhimuirum. Following Salmonella infection, NAIP KO mice had increased numbers of TCRαβCD8αα and TCRγδ+ intraepithelial lymphocytes, suggesting a possible source of TNF during barrier collapse. This effect may be driven by the increased bacterial burden in colon and mesenteric lymph node tissue we observed in NAIP KO mice. Using colonic organoids, we have also identified altered basal levels of prostaglandins (PGF2α) and IL-15/IL-15R complex, which could explain altered IEC proliferation and survival. Our ongoing studies aim to further evaluate the mechanisms and impact on mucosal immunity in response to inflammatory challenge.

P.21 **Fancy a cigarette? Addressing the need of an ethically sound mouse model to define the immunopathophysiological mechanisms of cigarette smoke-induced chronic obstructive pulmonary disease (COPD)**

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Chronic obstructive pulmonary disease (COPD) is a long-term and progressive respiratory condition most frequently caused by chronic cigarette smoking and characterised by marked pulmonary and systemic inflammation. As a disease, COPD places a significant burden on global medical and economic resources, and treatment revolves largely around symptom management and intervention during exacerbations. Murine models are frequently used to investigate key immunopathogenic mechanisms driving disease, yet despite being a topic of significant focus, findings from animal models are limited in their scope due to limited investigative techniques. As such our understanding of the role of immunity in COPD pathogenesis and persistence is relatively insufficient, and in addition most current models do not actively reflect human disease or the three Rs of animal research: replacement, refinement and reduction. Our research group therefore aims to develop a novel 3Rs friendly murine model of COPD using an intranasal cigarette smoke extract (CSE) exposure method to investigate the role of pulmonary and systemic inflammation on COPD pathogenesis. We have developed and validated an extensive 24 marker spectral flow panel and 18 gene RT-qPCR panel to thoroughly investigate the phenotypes and effector functions of numerous immune cell types and inflammatory genes known to play a role in COPD pathophysiology. Here we describe the step-by-step processes utilised and challenges faced to develop and validate the investigative techniques used for the model, as well as current findings from the beginning of our pilot study.

P.22 **Therapeutic STmΔaroA preferentially invade proliferating cells in human colorectal cancer**

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Attenuated Salmonella typhimurium (STm) home to and colonise tumours, where it induces tumour regression via mechanisms including metabolic competition, reducing tumour stemness and inducing an immune response. In this study, we aimed to further understand the mechanism by which attenuated STm specifically target tumour stem cells. Using a range of invasion machinery-deficient STm, coupled with pharmacological inhibitors, we identify type-3 secretion system component SipB engagement with host membrane cholesterol as essential for intracellular invasion of STm into tumour organoids. Previous studies have shown that cell-surface cholesterol is maximal during mitosis, and we show that STm preferentially invade ki67+ cells and blockade of proliferation abolishes STm invasion. Although SipB-deficient STm cannot invade intracellularly, we show that oral gavage of sipB-deficient STM into Apcmin/+ mice led to colonisation of polyps, and thus still have the potential to affect tumour metabolic landscape and immune infiltration. Bulk RNA-sequencing of patient-derived and mouse tumour organoids infected with STmΔaroA or STmΔaroA in combination with a cholesterol-sequestering compound allowed comparison of extracellular and intracellular bacteria on tumours in vitro. Intracellular infection led to upregulation of a range of chemokines and innate pathways, whereas extracellular bacteria induced enrichment of genes involved in metabolic pathways, which may drive differential immune responses in vivo. We propose that preferential invasion of fast-dividing cancer stem cells by Salmonella via SipB¬–cholesterol interaction diminishes the stem cell pool within the tumour, as well as altered chemokine production. We next aim to identify to what extent intracellular invasion is needed for therapeutic efficacy.

P.23 **Exploiting immune cells by Multiplex to improve the Immune score in colorectal cancer**

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Background: Immunoscore is a long-tested classification system for colorectal cancer (CRC), which relies on quantification of CD3/CD8 infiltration in tumor or invasive margin. However, this system only exploits 4 four parameters (two proteins, two tissue regions) to build classification due to the limitation of Immunohistochemistry so that other immune cells contribute to tumor immune network (such as macrophages, T help cells) have been ignored and tissue regions are limited. This study aims to define the immune neighborhoods and link these with patient's prognosis.
Method: Six different antibodies (CD4, CD8, CD34, epithelial cell adhesion molecule, CD68, MHC-II) are used for multiplex in 4000 tissue microarray (TMA) cores of 1000 CRC patients, which relies on Akoya multiplex kit/Leica Novolink detection system and Akoya Fusion platform. Akoya Inform was chosen as the machine learning software for tissue/cell segmentation and phenotype identification. The cytomap was used for unsupervised cluster and R for other bioinformatic analysis. All bioinformatics results will be evaluated based on clinical data of CRC.
Result and Conclusion: CD4+, CD8+, and CD68+ cells positively correlate with CRC patient survival. Unsupervised cluster reveals several clearly distinguished clusters in different CRC patients by combing CD34, epithelial cell adhesion molecule and MHC-II with immune cells together, which could possibly explain comprehensive immune network in CRC.

P.24 **Long-chain fatty acid uptake from the bone marrow microenvironment suppresses T cell function in Multiple Myeloma**

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Multiple Myeloma (MM) is a plasma B cell malignancy that develops in the bone marrow (BM) and is preceded by the asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS). Despite observations that T cell function is dysregulated in MM, checkpoint blockade therapies have not been successful, indicating that alternative mechanisms operate in the BM microenvironment to suppress T cell activity. Here, we assessed T cell phenotype, function and metabolism in matched peripheral blood (PB) and BM samples from healthy aged-matched donors and across the spectrum of MM disease progression. We identify that BM CD8+ T cell abundance and cytokine expression decrease with MM development, and furthermore, that CD8+ T cell function is consistently impaired within BM samples compared to matched PB, confirming suppression within the BM microenvironment. These changes were accompanied by decreased mitochondrial mass and an elevated capacity of T cells to take up long-chain fatty acids. In vitro approaches confirmed that uptake of BM lipids reduces BM CD8+ T cell mitochondrial mass and supresses cytokine expression, which were decreased by culture in autologous BM plasma but rescued by lipid removal. This work gives new insight into the pathology of MM disease and suggests new ways to restore T cell function of MM bone marrow.

P.25 **Optimising the multiplex immunohistochemistry images of macrophages in Breast Cancer**

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Background. Tumour-associated macrophages (TAMs) are one of the most abundant immune components in breast cancer (BC). The current understanding of the heterogeneity of phenotypic manifestations of different macrophage subpopulations indicates the need for simultaneous assessment of several specific macrophage markers, which increases the accuracy of identifying a particular macrophage subpopulation. This would allow us to identify novel prognostic and

predictive markers of cancer. Compared to Caucasian breast cancer patients, African women present with notoriously more aggressive breast cancer tumours, at a younger age, with diagnosis at a later stage and with a large number of triple-negative cases. These factors result in a higher mortality rate. However, despite the higher mortality rate, immune infiltration of African women's breast cancers is poorly studied.

Method. We use a Breast cancer TMA assembled from Ghanaian women, an African population to describe the development and optimisation of a seven-colour multiplex IHC panel, consisting of CD64, CD163, CD68, TREM-1, CD3, CK and DAPI in BC FFPE tissue.

Results. We identified many factors that influenced the quality of the acquired images. Macrophage subtypes can be divided into single-positive, double-positive, and negative-stained cells detected in both intra-epithelial and intra-stromal in BC.

Conclusion. This research indicated an optimal condition of the m stain for Nottingham and African BC, which will be applied for staining a TMA containing tumour cores from 2000 BC patients for Nottingham and 400 African BC patients. Then, the clinicopathological data from both the African and Nottingham cohorts will be compared.

P.26 **Maternal Vaccination against Streptococcus pneumoniae leads to long-lasting immunity to offspring**

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Maternal Vaccination to provide protection to the unborn infant is an attractive way to prevent infections in the early weeks and months of life. Despite this, the efficacy or mechanisms underpinning this approach are not well studied. The leading cause of child mortality by infection worldwide is Streptococcus pneumoniae (pneumococcus), particularly in low-to-middle-income countries (LMIC). This prevalence of infection could be due to vaccines (e.g. Prevnar13 (PCV13)) not being administered until the infant is 8 weeks old. Therefore, in children there is a window of susceptibility to this infection that could be addressed through maternal vaccination.

We have used novel mouse models of maternal vaccination (MV) with PCV13 and challenges using pneumococcus to identify the features of acquired immunity. Maternally vaccinated (MV'd) offspring acquired surprisingly long-lasting antibody responses as well as reduced bacterial burdens in the bronchoalveolar lavage (BAL), lung and blood suggesting protection against invasive pneumococcal disease is present. Moreover, MV'd offspring have distinct immune signatures associated with increase maternal cell populations. MV'd offspring display distinct enhanced levels of immune control in the BAL, increased maternal B and plasma cell populations in the lung and bone marrow as well as bone marrow cells that secrete PCV13-specific antibodies.

Therefore, MV suggests long-lasting immunity to infection and potentially has discrete impacts on host immune homeostasis.

P.27 **NRK1 fine-tunes NAD(P)/H, glutathione and reactive oxygen species abundance during CD4+ T cell activation to regulate their function and exhaustion**

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Activation of CD4+ T cells drives transcriptional and translational changes, accompanied by metabolic reprogramming to meet the bioenergetic demand. Nicotinamide Adenine Dinucleotide (NAD/H) and NAD phosphate (NADP/H) are key metabolic redox co-factors supporting this, and are linked to abundance of the antioxidant glutathione (GSH), through activity of the pentose phosphate pathway(PPP), which couples NADP/H oxidation to GSH/GSSG reduction. NAD can be synthesised de

novo or salvaged from nicotinamide (NAM), via nicotinamide mononucleotide (NMN), by NAM phosphoribosyl-transferase (NAMPT) –the major pathway in T cells. Recently, Nicotinamide Riboside Kinase (NRK)1 was identified to phosphorylate Nicotinamide Riboside (NR) to NMN, thereby directing it into the salvage pathway. Contribution of NRK1 to NAD(P)/H synthesis in T cells and its role in their biology has not yet been studied.

We observe NRK1 is upregulated in human and murine CD4+ T cells upon activation. In human cells, this is TCR- and CD28-dependent and requires ERK, NFAT, SRK, Pi3K and Akt activity. Provision of human CD4+ T cells with exogenous NR increases NAD/H and NADP/H as expected, particularly when NAMPT is inhibited. However, surprisingly, NR treatment of NAMPT-competent cells suppresses rather than supports cytokine secretion and prevents exhaustion. Consistently, CD4+ T cells from NRK1-deficient mice demonstrate decreased NAD/H abundance but increased activation and cytokine secretion vs. WT cells. T cell cytokine suppression by NR was associated with decreased reactive oxygen species (ROS) abundance, whilst NRK1-KO CD4+ T cells demonstrate increased ROS, alongside decreased GSH/GSSG ratios. Taken together, the data suggest NRK1 activity in CD4+ T cells "tops up" NAD(P)/H levels generated by NAMPT to maintain optimal GSH abundance and control of ROS. In its absence, increased ROS signalling promotes T cell activation, cytokine secretion and exhaustion. Consistent with this, PPP inhibition prevents suppression of IFN-γ by NR.

P.28 **Neuroimmunology of cryptococcal meningitis: exploring the roles of CNS-localized myeloid cells in T-cell responses**

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The recruitment and activation of CD4 T-cells within the CNS during infection is only partially understood. We have used a model of cryptococcal meningitis, a lethal fungal neurological infection that is dependent on CD4 T-cells for protection, to delineate the cell populations and events regulating entry of CD4 T-cells to the fungal-infected brain. We developed a novel TCR transgenic mouse model (CnTII-Tocky) to analyze anti-fungal CD4 T-cell responses and the role of myeloid cells in these responses. This model reports Nr4a3-dependent TCR signaling activity and cytokine production of Cryptococcus-specific CD4 T-cells during in vivo infection, enabling us to correlate T-cell numbers, proliferation, fungal burden, TCR signaling and cytokine production in the same animal. Using these mice, we show that fungal-specific CD4 T-cells only migrate into the CNS during acute meningitis but not chronic infection. Infiltrating CD4 T-cells had undergone rounds of division, produced IFNγ and did not engage their TCR as they expressed low TCRβ and reduced Nr4a3-dependent TCR signaling. Acute meningitis induced high numbers of inflammatory CXCL10+ macrophages. Single-cell RNA-seq analysis of macrophages revealed significant heterogeneity however antigen presentation and T-cell engagement pathways were largely localized to one population that shared characteristics with border macrophages. In contrast, CNS-resident microglia did not significantly upregulate genes involved with T-cell activation and appeared relatively unchanged in both acute and chronic CNS infection models. Taken together, our results show that monocyte-derived macrophages infiltrate the CNS during acute fungal infection and produce CXCL10 to recruit IFNγ-producing T-cells, which have limited TCR-dependent interactions with CNS-resident myeloid cells. In contrast, chronic infection suppresses the development of the macrophage population for CNS recruitment of T-cells. Taken together, we have delineated a complex series of events that regulate CD4 T-cells entry to the CNS during cryptococcal meningitis, revealing several novel targets for immune-based therapies for this life-threatening infection.

P.29 **Immature granulocyte counts and red cell distribution width may be used to predict survival outcome in alcoholic hepatitis**

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Introduction: Alcoholic hepatitis (AH) is an acute inflammatory form of alcohol-related liver disease, with significant short-term mortality when severe. Prediction of which patients will do poorly

remains a clinical challenge. In this study we use readily available automated haematology analysers to investigate whether blood cell parameters help in the diagnosis of AH, and can identify which patients have the highest short-term mortality risk.

Methods: In this prospective study, blood samples were obtained from inpatients with alcoholic hepatitis (AH) (n=39), patients with alcohol-related liver cirrhosis, including abstinent patients (ALD-A) (n=39) and those that were actively drinking (ALD-D) (n=11). Samples were run on the Sysmex-XN 1000 haematology analyser. Patient demographics and clinical outcomes (disease severity, as measured by the Glasgow Alcoholic Hepatitis Score (GAHS), and survival up to 1 year) was obtained from electronic hospital records and compared to healthy controls (n=39).

Results: Patients with AH had significantly elevated immature granulocyte (IG) counts, compared to HC ($p<0.0001$), ALD-A ($p<0.0001$) and ALD-D ($p=0.04$). AH patients also had increased red-cell distribution width (RCDW), compared to HC ($p<0.0001$), ALD-A ($p<0.0001$) and ALD-D ($p=0.0040$). In AH, IG counts and RCDW both correlated with GAHS ($r= 0.4323$, $p=0.0075$ and $r=0.6315$, $p<0.0001$). Patients who died by 28 days follow up had significantly higher immature granulocyte counts ($p<0.00001$) and higher RCDW ($p=0.008$) than patients who survived.

Conclusion: IG counts and RCDW, easily obtained on automated haematology analysers, help distinguish patients with AH and could identify patients at highest risk of short term mortality to help inform clinical management strategies.

P.30 **Towards a B cell Vaccine for Tumour expressed Self-Antigens**

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Targeting tumour antigens is mayor challenge in cancer-immunotherapy. We try to use vaccination to induce antibodies targeting self-antigens expressed on tumour vessels. We have shown that a conjugate vaccine can induce autoantibodies specific to the tumour endothelial antigen Robo4, which is selectively expressed on tumour vascular endothelium, but not or low on healthy vasculature. By linking the extracellular domain of Robo4 to a common carrier protein, autoreactive B cells could recruit T cell help specific for the carrier. This protocol could efficiently induce specific anti-Robo4 antibodies and suppress tumour growth in a Lewis Lung carcinoma (LLC1) mouse model. This project aims to optimise the vaccine-induced antibody response to Robo4 in LLC1 tumours and understand how the vaccine breaches immune tolerance in cancer.

As most patients have pre-existing T cell memory to tetanus, we decided to use the non-toxic fragment C of tetanus toxin (TTc) as the carrier protein. Our data shows that vaccination of mice with Robo4-TTc in adjuvant can efficiently induce the production of Robo4-specific antibodies, particularly IgG1. Moreover, the injection of Robo4-TTc retarded the tumour growth in a LLC1 model. Further we discovered increase in NK cell, CD4+, and CD8+ T cell and dendritic cell populations in tumours after Robo4 vaccination. LLC1 is a non-inflamed, poorly immunogenic tumour, resistant to immune-checkpoint therapy (ICT). Our data indicate that this vaccination strategy may have potential to improve the results of cancer therapy for ICT-resistant “cold” tumours.

Keywords: Cancer-immunotherapy, Vaccine, Robo4, Lung cancer.

P.31 **SARS-CoV-2 variant infection of liver epithelial cells**

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SARS-CoV-2 is the coronavirus responsible for the 2020 COVID-19 pandemic, and commences infection in the human respiratory tract. We now appreciate that there are multiple anatomical sites that harbour infection beyond the lungs, causing significant pathology. During infection, features of transient liver damage have been reported, as measured by peripheral blood liver enzymes released by damaged hepatocytes. In most patients, liver injury resolves after recovery. However, the long-term impact of infection in the liver has not been investigated. Furthermore, it is suggested that

SARS-CoV-2 infection may sensitise the liver for increased damage following other infections, such as adenovirus. To study the impact of SARS-CoV-2 variants of concern on liver pathobiology, we developed a high content immunofluorescence-based assay in hepatoma cell lines. Huh-7 and interferon pathway RIG-I deficient Huh-7.5 cells were characterised for susceptibility and permissibility to the ancestral strain, and variants of concern Alpha, Delta, Omicron BA1 and Omicron BA2 that have been widely spread in the UK over the past three years. Our data showed that hepatoma cell lines were susceptible and supported SARS-CoV-2 replication despite less than those of human lung epithelial cell line A549-ACE2 and the gold-standard cell line for coronavirus infection, Vero cells. This was explained by the low ACE2 receptor expression in the hepatoma cell lines. Overall, our model is suitable to study infection, syncytia formation and variant neutralisation in liver, lung, and kidney epithelial cells, and could be adapted to test emerging variants of SARS-CoV-2 in other human and non-human adherent cell lines.