Bridging the gap between clinical and academic immunologists in the Midlands

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Abstracts

Selected short talks

Harnessing enclysis to control Treg cell frequencies in the liver tumour microenvironment

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Regulatory T cells (Treg) are anti-inflammatory cells that contribute to the liver's natural immunotolerance. Treg cell abundance perturbs anti-tumour surveillance in the liver, hinders cancer targeting by host immunity and reduces the impact of immunotherapy. We previously reported that hepatocytes delete live Treg cells by enclysis. We hypothesised that increasing enclysis has the potential to boost anti-cancer immunity specifically in the liver.

We developed high content imaging-based co-culture assays to screen FDA approved drugs for enclysis-modulating effects. Promising targets were validated in human liver slices ex vivo. Biomarkers for enclysis competence were assessed by immunohistochemistry using non-cirrhotic human livers rejected for transplantation, and explant livers from end stage diseases of metabolic, autoimmune or viral aetiologies.

The FDA approved drug screen revealed new molecular targets for the metabolic regulation of hepatocyte enclysis. Herein we describe the role of Cyclooxygenase-2 (COX-2) in regulating enclysis in vitro and in liver tissues ex vivo. Pre-treatment of hepatocytes with COX-2 inhibitors aspirin, and the more specific celecoxib, increased enclysis. The opposite occurred when hepatocytes were treated with the COX-2 enhancer prostaglandin E2. Intracellular COX-2 expression could be toggled in hepatocytes from liver slices ex vivo, yielding new opportunities for biomarkers predicting enclysis competence in tumours and their microenvironment.

We show that it is possible to target enclysis pharmacologically, to modify Treg cell frequencies in the liver. These data partially explain the growing literature on the beneficial effects of aspirin in HCC prevention and immunotherapy and provide proof of concept for enclysis modulation in immunotherapy.

Investigating Functional abilities of Exhausted and Senescent T cells in DLBCL

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Despite significant advances in the treatment of relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) with the introduction of T cell directed therapies including chimeric antigen receptor (CAR-T) T-cells and CD20xCD3 bispecific antibodies (BsAbs), approximately 50% of patients either fail to respond or relapse to these therapies. Both T cell exhaustion and senescence are two distinct states that may have a critical role in determining outcome through defective effector functions, impaired proliferation, and cell cycle arrest. We investigated the impact of these dysfunctional states on T cell phenotype and BsAb killing using an in vitro model.

Initial experiments found senescence can be induced by repeated exposure to anti-CD3/CD28 antibodies over 4 weeks. These cells initially begin to express exhaustion markers at weeks 1-3, with T cells expressing increasing levels of inhibitory receptors PD1, LAG3, TIM3 and CTLA4. However, these are lost and replaced by expression of senescence markers by week 4, exhibiting loss of CD27 and CD28 alongside gain of CD57 and KLRG1. This senescent state was confirmed by observing the complete loss of proliferation at week 4.

B cell killing decreased as exhaustion markers increased, beginning at 80.45% at week 0, to 0.13% at week 3. However, at week 4 when cells instead express senescence markers, a small amount of killing ability was regained by the T cells (13.8%), leading to an increase in B cell death. This data obtained from the positive ‘high responder’ DLBCL cell line was also reproducible and in the resistant negative control.

Intrinsic Folding Properties of the HLA-B27 Heavy Chain Revealed by Single Chain Trimer Versions of Peptide-Loaded Class I Major Histocompatibility Complex Molecules

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Peptide-loaded Major Histocompatibility Complex (pMHC) class I molecules can be expressed in a single chain trimeric (SCT) format, composed of a specific peptide fused to the light chain beta-2 microglobulin (β2m) and MHC class I heavy chain (HC) by flexible linker peptides. pMHC SCTs have been used as effective molecular tools to investigate cellular immunity and represent a promising vaccine platform technology, due to their intracellular folding and assembly which is apparently independent of host cell folding pathways and chaperones. However, certain MHC class I HC molecules, such as the Human Leukocyte Antigen B27 (HLA-B27) allele, present a challenge due to their tendency to form HC aggregates. We constructed a series of single chain trimeric molecules to determine the behaviour of the HLA-B27 HC in a scenario that usually allows for efficient MHC class I molecule folding. When stably expressed, a pMHC SCT incorporating HLA-B27 HC formed chaperone-bound homodimers within the endoplasmic reticulum (ER). A series of HLA-B27 SCT substitution mutations revealed that the F pocket and antigen binding groove regions of the HLA-B27 HC defined the folding and dimerisation of the single chain complex, independently of the peptide sequence. Furthermore, pMHC SCTs can demonstrate variability in their association with the intracellular antigen processing machinery.

Analysing publicly available datasets to understand macrophage transcriptome reprogramming during M.tb infection

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Tuberculosis has the highest mortality of any infectious disease and causes significant social and economic burden. On inhalation, Mycobacterium tuberculosis (Mtb) enters the respiratory system and primarily infects alveolar macrophages. Mtb infection changes host transcriptomes. Identifying differentially expressed genes helps identify host immune pathways induced by Mtb infection, showing which pathways can be targeted to improve tuberculosis prognosis. Mtb induces metabolic reprogramming in infected macrophages, promoting glycolysis and increasing lactate production. Lactate itself has newly discovered signalling properties and can be sensed and uptaken by macrophages.

We have analysed publicly available transcriptomics datasets from human alveolar macrophages and monocyte-derived macrophages to identify genes common to the immune response against Mtb in the human lung. These cell types had distinct transcriptomes, highlighting the importance of working with primary human cells. We found two consistently upregulated genes: PTGS2, encoding COX-2 which produces prostaglandins from arachidonic acid, and CXCL8, encoding IL8, a pro-inflammatory cytokine and neutrophil chemokine. GPR132, encoding a GPCR for which lactate is a ligand and regulates downstream signalling, was upregulated in some cases. We have investigated the relationships between these proteins using in vitro alveolar macrophage models to elucidate their function in inflammatory signalling.

Understanding the relationships between these three proteins could help to develop novel HDTs. GPCRs are common therapeutic targets, with roughly 35% of all medicines targeting GPCRs. Novel HDTs may be developed to target GPR132, influencing the relationship between the three proteins in order to produce a protective effect to the host during diseases involving chronic inflammation.

Poster presentations

P.01 **Proteomic analysis of ex vivo glioblastoma tumour derived extracellular vesicles reveals several immunomodulatory candidates**

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Introduction
Glioblastoma (GBM) is the most aggressive and common brain cancer, accounting for 32% of primary brain tumours in England. It has poor prognosis, with average survival around 15-months and 5-year survival below 10%. One reason for this is immunosuppressive remodelling of the tumour microenvironment, which is mediated through several mechanisms, including GBM-derived extracellular vesicles (GBM-EV). We aimed to uncover protein candidates present in ex vivo GBM-EV which may contribute to immunosuppressive remodelling.

Methods
Serum-free transport medium from 58 excised GBM tumours was collected, and the GBM-EV were extracted through size-exclusion chromatography. Molecular subtyping was performed via qRT-PCR to classify the tumours (16 classical, 13 proneural, 3 mesenchymal, 26 not classified). Proteins were extracted from GBM-EV, separated by gel electrophoresis, and analysed by liquid chromatography tandem mass spectrometry.

Results
1,397 proteins were identified with 1,167 above a set abundance threshold. Gene ontology analysis showed enrichment in cell growth-, metabolic-, and transport-related biological processes. When separating by molecular subtype, all but 6 proteins were present in all three subtypes and gene ontology analysis was similar between subtypes, suggesting GBM-EV have similar profiles independent of subtype. There were 38 differentially expressed proteins which showed subtype clustering in principal component analysis, suggesting the GBM-EV proteome can determine subtype. Further, gene ontology analysis identified 27 membrane proteins on GBM-EV with an immune function.

Conclusions
Analysis reveals several immunomodulatory candidate proteins within ex vivo GBM-EV. This is the largest proteomic analysis of ex vivo GBM-EV to-date and may identify novel treatment targets in GBM.

P.02 **Epithelial-derived EVs as modulators of immune response to smoking and vaping**

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Cigarette smoke is a well-established risk factor in the development of many respiratory and cardiovascular diseases. The long-term effects of e-cigarettes still need better understanding, especially as these devices are becoming increasingly popular, particularly among young people. The lung epithelial layer is the first line of defence against inhaled particles and pathogens from the external environment, such as tobacco smoke and vape, playing a critical role in the maintenance of immune homeostasis between the airway epithelium and the environment, or in the development of inflammation. Extracellular vesicles (EVs) are small, membrane-enclosed vesicles that can be secreted by every type of cell in the human body. These vesicles act as important mediators of intercellular communication, capable of transferring biomolecules between cells, thus influencing cell function. Because of their function, the role of EVs on the development of chronic lung inflammation has become a target of increasing interest, but little is known about the effect of conventional smoke and e-cigarette vape on lung EVs, and their downstream effect on the development of lung inflammation.

This PhD project aims to understand the role of lung epithelial-derived EVs on the immune system after exposure to conventional smoke and electronic cigarette vape. Human epithelial cells are exposed to Cigarette Smoke Extract (CSE) or Electronic Cigarette Vapour Extract (ECVE) and both the cytokines (Interleukin-6 [IL-6] and Interleukin-8 [IL-8]) and EVs produced are examined. Interestingly, we show that the presence of the filter on a conventional cigarette has a greater influence than without the filter.

P.03 **Understanding the role of the vaginal microbiome in the maternal innate immune system**

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Host-microbiome interactions have become a popular talking point in recent years and a hallmark of women's health is a Lactobacillus crispatus- dominated vaginal microbiota. Conversely, a high diversity microbiota consisting of a more even proportion of bacterial species, which are commonly associated with bacterial vaginosis, can lead to several health issues such as increased risk of sexually transmitted infections. An association between a high diversity microbiota and premature birth has been found, potentially arising from the activation of toll-like receptors 2 and 4 by bacterial ligands which result in a pro-inflammatory response, inducing pro-labour gene expression. The outermost layer of L. crispatus, a proteinaceous 2D crystalline array known as the S-layer, composed of multi-functional surface layer proteins, plays a key role in host microbiota interactions. Interestingly, surface layer protein glycosylation allows for binding to the anti-inflammatory C-type lectin, DC-SIGN. Identification of these DC-SIGN glycan ligands can help us understand the differences observed between maternal inflammatory states and hopefully lead to the development of probiotics to treat women at risk of preterm birth and other adverse reproductive health outcomes caused by a high diversity microbiota.

P.04 **Investigating the impact of lactate on alveolar macrophages in tuberculosis disease**

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Tuberculosis remains the deadliest infectious disease, 10 million people are infected, and it kills 1.5 million people annually. The effectiveness of current treatments is threatened by multidrug resistance therefore there is a need to develop new host-directed therapies. Mycobacterium tuberculosis (Mtb) is the causative agent of TB. Upon entry into the lungs Mtb is engulfed by alveolar macrophages (AMs) which alert the innate immune system. Host-pathogen interactions bring about a metabolic reprogramming leading to increased glycolysis and an accumulation of lactate in the lungs. Little is known about the implications of this increase in lactate on the function and metabolism of AMs.

I aimed to model this lactate rich environment and study how lactate impacts AMs, by subjecting them to phloretin, a lactate transporter inhibitor and lactate sensor GPR132 agonists and antagonists. I demonstrated by flow cytometry that AMs express lactate transporters MCT1 and MCT4. By ELISA, I demonstrated that lactate treatment leads to increased IL-8 secretion, which is reversed by blocking lactate transporters and lipid droplet formation inhibitors. Antagonising GPR132, a lactate sensing G protein coupled receptor also decrease IL-8 secretion, suggesting GPR-132 mediates IL-8 release. Antagonising GPR132 decreased lipid droplet formation, suggesting lactate signals through GPR132 to stimulate increase lipid droplet formation which increases IL-8 release. This work suggests a mechanism by which AMs respond to lactate by releasing IL-8, a potent neutrophil chemoattractant which may help clear Mtb. This work aims to further our understanding of host-tb interactions towards to development of a new host directed therapy.

P.05 **Immunoprofiling gd T-cells present in matched human blood and tissue samples of colorectal cancer patients.**

Annabelle Bennett, Priyanka Chevour, Martin Davey

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Colorectal cancer is the second leading cause for cancer-associated mortality worldwide. Due to it being the third most diagnosed cancer in the UK, there is a vital urgency to find new immuno-therapeutic targets.

$\gamma\delta$ T-cells have been implicated in tumour immunity, however unlike conventional T-cell research, their role remains largely unknown. We hypothesise that clonally expanded tumour infiltrating $\gamma\delta$ T-cells have distinct effector-like, tissue-resident and cytolytic phenotypes that drive antigen-specific immune responses within the TME.

Spectral flow cytometry was conducted on matched human PBMCs, normal adjacent tissue (NAT) and tumour tissue samples from colorectal cancer patients to ascertain expression of 33 different markers on the surface of $\gamma\delta$ T-cells present. Primarily, Vδ1s were observed to be the principal $\gamma\delta$ T-cell located in colorectal tissues.

Preliminary results suggest that higher populations of CD27-CD45RA- Vδ1s are found in NAT and tumour tissues compared to PBMCs in matched samples. These CD27-CD45RA-Vδ1+ cells display tissue-associated markers including CD103, CD69, and CX3CR1. Further analysis reveals that this subpopulation also express NKp46, an NK receptor associated with gut-resident IELs.

Additional preliminary results suggest higher CTLA4 expression in Vδ1+ T-cells in both NAT and tumour tissue than matched PBMCs, with NAT samples expressing higher CTLA4 than tumour samples. Reduced CTLA4 expression was observed in effector-like Vδ1s compared to naïve-like Vδ1s in all matched samples, indicating an adaptive phenotype of these Vδ1 T-cells, though remains largely unexplored. Determining marker expression of potential anti-tumour effector-like $\gamma\delta$ T-cells could provide potential groundwork for future research on therapeutics for colorectal cancer.

P.06 **Immunoprofiling unconventional $\gamma\delta$ T-cells in colorectal cancer**

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Colorectal cancer is the second leading cause for cancer-associated mortality worldwide. Due to it being the third most diagnosed cancer in the UK, there is a vital urgency to find new immunotherapeutic targets. γδ T-cells have been implicated in tumour immunity, however unlike conventional αβ T-cells, their roles in tumour immunity remain largely unknown. We hypothesise that clonally expanded tumour infiltrating γδ T-cells have distinct effector-like, tissue-resident and cytolytic phenotypes that may drive antigen-specific immune responses within the tumour microenvironment (TME).

33-parameter full spectrum flow cytometry was conducted on matched blood, normal adjacent tissue (NAT) and tumour tissue samples from patients undergoing colorectal tumour resection surgery at the University Hospital Coventry and Warwickshire (UHCW).

We find in the blood that γδ T-cells, normally expressing Vγ9/Vδ2 T cell receptors, are often skewed towards Vδ1+ T-cells expressing CX3CR1 and CD45RA, consistent with a shift towards an effector-like phenotype. Within matched NAT and tumour tissue, we predominantly find CD27-CD45RA-CD103+CD69+NKp46+ Vδ1 T-cells (Vδ1tissue) compared to blood, consistent with a tissue resident population. Further analysis revealed that Vδ1tissue cells had reduced CTLA4 expression in tumours compared to NAT yet had elevated PD-1 expression in the tumours, indicating an interesting link between immune checkpoint markers and Vδ1tissue cells in colorectal cancer.

Determining the potential anti-tumour tissue-associated γδ T-cells could provide potential new immunotherapeutic targets for colorectal cancer.

P.07 **T cell phenotypic changes following the first cycle of gemcitabine, cisplatin and anti-PD-L1 in cholangiocarcinoma**

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Introduction
Cholangiocarcinoma is a rare biliary tract cancer with high mortality rates. First-line palliative treatment includes gemcitabine, cisplatin, and anti–PD-L1 agent, durvalumab, however response rates vary widely. Reliable biomarkers to predict efficacy or adverse events are lacking. This study aimed to analyse peripheral blood T cell phenotypes in patients with cholangiocarcinoma.

Methods
Peripheral blood samples were obtained at baseline and following the first cycle of gemcitabine–cisplatin–durvalumab therapy from consenting patients with advanced cholangiocarcinoma. PBMCs were isolated and cryopreserved before analysis using a 30-colour spectral cytometry panel to identify major T cell phenotypes.

The primary objective was to identify correlations between distinct T cell phenotypes and the following clinical response metrics: overall survival, progression-free survival and frequency of immune-related adverse events (irAEs).

Results
The cohort consisted of 13 patients (54% male), with a median age of 68.5 years (range 33–80). 6 had intrahepatic cholangiocarcinoma; 4 extrahepatic cholangiocarcinoma; 3 gallbladder cholangiocarcinoma. The median overall survival was 10.5 months [95% CI: not estimable].The median progression-free survival was 5.8 months [95% CI: 3.6-not estimable] and 6 of the patients presented with irAEs. Unsupervised clustering identified a CD4+ T cell subset that was associated with pharmacodynamic response and a second cluster that significantly increased on therapy in patients exhibiting irAEs.

Conclusion
To our knowledge these findings are the first analysis of peripheral blood T cell dynamics in cholangiocarcinoma patients receiving triple therapy. They demonstrate that changes in peripheral blood T cell phenotypes may be useful correlates of important clinical outcomes.

P.08 **Interrogating metabolic control of macrophage immune function by TNF-α**

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Anti-TNF-α therapies have revolutionised treatment of chronic inflammatory conditions. However, TNF-α is also important in initiating and maintaining inflammatory responses to pathogens; this is highlighted by the fact that anti-TNF-α treatment increases the risk of reactivating latent mycobacterium tuberculosis. Our group identified that TNF-α promotes

metabolic reprogramming of CD4+ T cells upon activation, supporting differentiation and function. We hypothesise this may also occur in macrophages, which are important for control of latent tuberculosis. We have interrogated this in human monocyte-derived macrophages, stimulated with lipopolysaccharide (LPS) in presence of isotype control or TNF-α neutralising antibody. Our findings indicate that blocking cell-intrinsic TNF-α signalling reduces macrophage surface expression of costimulatory and antigen-presenting surface molecules (CD40 and MHC class II) and reduces production of the key cytokines IL-6 and IL-10, but has little impact on mitochondrial function or glycolysis. Conversely, we observed that blocking TNF-α signalling prevented LPS-induced changes in several enzymes within NAD/H synthesis pathways, including IDO1, KYNU, QPRT and NAMPT. NAD/H is a coenzyme and cofactor with a key role in cellular and systemic metabolism and is phosphorylated to NADP/H which importantly controls cellular reactive oxygen species and is important for pathogen killing. Whilst a role for TNF-α in regulating IDO1 expression is well-documented, effects on other parts of these pathways have not been previously described. We now plan to investigate how alterations in these pathways affect macrophage metabolism and function, by employing approaches including stable isotope-based metabolic tracing of tryptophan, assessment of cellular NAD(P)/H status, phagocytosis and mycobacterial control.

P.09 **The impact of ruxolitinib and baricitinib on IL-6 evoked phospho-STAT3 induction across human immune cell subsets**

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Activation of intracellular signalling pathways by multiple cytokines, including those elevated in autoimmune and inflammatory disease such as IL-6, IL-11 and IL-22, result in the phosphorylation of STAT3 that may involve the JAK family of non-receptor tyrosine kinases, which includes JAK1, JAK2 and Tyk2. An understanding of this cell biology has led to the development of JAK inhibitors as useful therapeutic approaches to treat autoimmune and inflammatory disease.

Phosphoflow enables quantitative and high-throughput measurement of target proximal signalling events and is informative for the drug discovery pathway from early screening to clinical studies. We have developed and optimized phosphoflow cytometry assays to quantify agonist-induced phospho-signalling. In the present study we report IL-6 evoked phospho-STAT3 across immune cell subsets in PBMCs from healthy donors (e.g. CD4 T cells, CD8 T cells, NK cells, NKT cells, Tregs, γδ T cells, B cells, monocyte subsets) in order to compare the impact and pharmacology of two small molecule JAK inhibitors, ruxolitinib and baricitinib. Our results demonstrate differential sensitivity of immune cell subsets to IL-6 evoked pSTAT3 phosphorylation, and we present the sensitivity of this response to inhibition by the clinical utilised JAK inhibitors, ruxolitinib and baricitinib.

P.10 **Comparative in vitro efficacy of CD20xCD3 bispecific biosimilar constructs against diffuse large B cell lymphoma (DLBCL) cell lines with different levels of expression of CD20.**

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CD20xCD3 bispecific antibodies (BsAb) have shown unprecedented activity in relapsed/refractory Diffuse Large B Cell Lymphoma (DLBCL). However, there is currently no direct comparative evidence to inform construct selection in the clinic. Using biosimilar versions of glofitamab, epcoritamab, odronextamab and mosunetuzumab we compared their activity against a panel of DLBCL cell lines.

BsAb activity was assessed using a flow cytometry-based cytotoxicity assay. DLBCL cell lines with varying levels of CD20 expression – SU-DHL-10, UoL-RAD, Karpas-1718 and UoL-AME were treated with BsAb biosimilar (Proteogenix) for 24 hours, using healthy volunteer peripheral blood mononuclear cells as effector cells. B cell depletion (BCD) was calculated and effector T and natural killer (NK) cell activity determined.

All CD20xCD3 biosimilar constructs demonstrated dose-dependent killing against UoL-RAD and SU-DHL-10, with simultaneous activation and degranulation of T cells, but not NK cells. Glofitamab biosimilar demonstrated the highest activity against UoL-RAD and SU-DHL-10 (EC50 2pM and 59pM respectively). Comparative EC50 values for epcoritamab were 71pM and 537pM, for odronextamab 65pM and 129pM and mosunetuzumab 156pM and >10,000pM respectively. Despite low expression of CD20, UoL-AME was sensitive to glofitamab and odronextamab (EC50 802pM and 3248pM). Karpas-1718 was resistant to all biosimilar BsAb tested (EC50 >10,000pM).

These experiments suggest that glofitamab biosimilar had superior efficacy against DLBCL cell lines compared to other CD20xCD3 biosimilars, perhaps due to bivalent CD20 binding. Interestingly, significant responses to glofitamab were observed in vitro for UoL-AME, despite low CD20 expression. The biological basis of inherent resistance to all CD20xCD3 BsAbs seen in Karpas-1718 requires further investigation.

P.11 **The leukaemia drug asparaginase has potent suppressive effects of T cell function**

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Asparaginase (ASNase) is the cornerstone treatment for acute lymphoblastic leukaemia (ALL) and is also being combined with T cell immune checkpoint blockade (ICB) in phase I clinical trials for lymphoma. ASNase deprives tumours of the amino acid asparagine (Asn), thereby driving metabolic collapse. However, T cells harbour near-identical metabolic requirements, and so we questioned whether this drug would have deleterious effects on T cell activation. Indeed, ASNase is poorly effective in adult populations, necessitating further understanding of its mechanism of action.

We employed dual T cell receptor (TCR) and Ifng reporter mice to interrogate the effects of ASNase treatment on T cell function. ASNase exerted potent suppressive effects on bulk CD4/CD8 T cell function in a dose-dependent manner, inhibiting both TCR signalling and Ifng production upon activation. This correlated with a reduction in the Asn sensor c-Myc, as well as decreased phosphorylation of Erk/mTOR/PI3K. ASNase-treated T cells also failed to up-regulate the Asn synthesis enzyme, asparagine synthetase (Asns). Intriguingly, cell sorting of memory subsets revealed that effector memory CD4 T cells (CD44+/CD62L- TEM) were more resistant to ASNase treatment, possessing elevated levels of Asns and robust Ifng transcription. In addition, in contrast to naïve T cells, TEM also upregulated clinically relevant immune checkpoints upon activation with ASNase treatment.

In summary, we show that ASNase—whilst a potent tumouricidal drug—may have differential impact on naïve versus memory CD4+ T cell subsets. These findings may be important in rationally designing T cell-targeting ASNase co-therapies incorporating immune checkpoint blockade.

P.12 **Investigation of the Bone Marrow Tumour Microenvironment in Acute Myeloid Leukaemia**

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Leukaemia is a group of blood cancers marked by uncontrolled proliferation of hematopoietic precursor cells within bone marrow (BM). AML progression is influenced by the bone marrow tumour microenvironment (BMME), where bone marrow-derived mesenchymal stem cells (BM-MSCs) regulate haematopoiesis and immune homeostasis. Pro-inflammatory signalling contributes to AML progression/niche modelling/immune suppression. In AML, BM-MSCs exhibit functional alterations, likely driven by unknown post-transcriptional mechanism. S100 proteins, which act as pro-inflammatory mediators in immune system, are dysregulated in AML, causing ineffective haematopoiesis and drug resistance.

This study investigated how AML cells and S100 proteins modulate BM-MSC function. BM-MSCs were co-cultured with AML cell lines (HL-60 and MOLM-13) or treated with S100 proteins to assess changes in proliferation (XTT assay), osteogenic differentiation (calcium assay), and cytokine secretion (immune activation suppressor IL-10 and immune activation promoter IL-6 via ELISA). Mass spectrometry identified S100 protein sources.

AML co-culture inhibited MSC proliferation, most prominently with HL-60 cells. S100 proteins did not alter proliferation. Osteogenic differentiation was reduced in AML co-cultures, largely due to MSC loss. Similarly, S100 proteins impaired differentiation through a different pathway. AML co-cultures decreased IL-10 secretion, while both AML cells and S100 proteins increased IL-6 levels, indicating a pro-inflammatory shift in MSC phenotype. MSCs retained S100 proteins intracellularly, while AML cells secreted them extracellularly.

These findings suggest that AML cells and S100 proteins differentially reprogram MSCs to alter their immune modulative function. Further studies will determine mechanisms of MSC abnormalities in AML and the involvement of S100 proteins to target them.

P.13 **Levels of CD20 expression do not determine response to Glofitamab in B cell lymphoma cell line models.**

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CD20xCD3 bispecific antibodies (BsAbs) have shown transformative single agent activity in B-cell malignancies. Glofitamab is unique as it retains bivalent CD20 binding. Nevertheless, 30-40% of patients fail to respond or relapse early. Acquired loss of CD20 expression underlies resistance in some cases but overall, molecular mechanisms of inherent and acquired resistance to CD20xCD3 BsAbs remain poorly understood. Here, using a panel of 42 B cell lymphoma cell lines with quantified levels of CD20 expression, we determined the ability of Glofitamab to induce malignant B cell death (BCD) in vitro using normal, healthy donor T/NK cells. Cytokine release, and changes in T and NK cell phenotypes were also assayed and compared with results with the monospecific Type 2 CD20 antibody, Obinutuzumab.

41/42 cell lines expressed CD20, ranging from 47 to 7.9 x 105 molecules/cell. BCD induced by Glofitamab was significantly higher than that observed with Obinutuzumab in 32/41 cell lines. Levels of CD20 expression were not correlated with Glofitamab-induced BCD; 9/42 cell lines expressed <5000 molecules/cell CD20 but 3 retained marked Glofitamab sensitivity (Mean IC50 33pM). Comparable heterogeneity was observed in 33 cell lines expressing >104-106 CD20 molecules/cell, with 26 responding (Mean IC50 17pM). One cell line expressing 5.3 x 104 CD20 molecules/cell was totally resistant. In Glofitamab low-responding/resistant cell lines, T cell activation and degranulation were preserved, indicating an inherent block to cytotoxic T-cell mediated lysis. These data indicate heterogeneity in B cell responses to Glofitamab, independent of CD20 expression; the molecular basis of this heterogeneity remain unknown.

P.14 **Elucidating T Cell Signalling with Optogenetics and Biosensors**

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Our immune system relies on T cells making appropriate and robust decisions to protect healthy cells whilst destroying pathogens or cancerous cells. To achieve this behaviour, extracellular cues are converted into information through biochemical reactions within the cell. These reactions, such as phosphorylation driven by kinases, integrate to form a signalling network that drives the overall functional outcome. Co-inhibitory receptors provide signals that suppress T cell activation and have thus gained clinical interest as therapeutic targets for diseases such as cancer. Current methods for probing the signalling network lack the resolution to further our understanding of the decision-making processes used by T cells, as well as the mechanism of action for co-inhibitory receptors. To address this limitation, we have developed

a toolkit of ratiometric fluorescent biosensors to probe key parts of the T cell signalling network with spectral flow cytometry, with the capacity for multiplexed measurements of pathways simultaneously. We have combined these biosensors with new optogenetic receptors to precisely control both T cell activation and inhibition through illumination with blue light. These synthetic, light-controllable receptors provide greater control and higher temporal resolution over cellular inputs, allowing for more thorough exploration of signalling dynamics. We have started to investigate the effect of PD-1 function on T cell activation and expect our approach to provide new insights into how signalling dynamics are decoded by the T cell signalling network more generally. These fundamental mechanisms should also help in the clinic, designing more efficient checkpoint inhibitors or combatting exhaustion during CAR-T therapy.

P.15 **Observing the cancer microenvironment pre- and post- immune checkpoint blockade in vivo**

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Introduction

Immune checkpoint inhibition works through blocking negative immune signals. PD-L1 over-expression is seen in cancer, dampening T cell responses through PD-1. Mycosis fungoides is a rare skin T-cell lymphoma and is unusual in that it can express PD-L1 and PD-1. We investigated the microenvironments (TME) response to therapeutic PD-L1 blockade in the EORTC-1652-CLTF trial.

Methods

Blood and skin biopsies were taken from patients with mycosis fungoides stage IIb-IV who received atezolizumab 1200mg IV every 3 weeks. Samples were taken at: pre-treatment (BLI), 5 days after (CY1), and pre-cycle 2 (CY2). Samples were analysed using mass-cytometry and correlated with clinical outcomes.

Results

Eleven patients provided 52 samples. Langerhans cells were the main expressors of PD-L1 in the TME. Treatment decreased apparent PD-L1 expression. There was a significant increase in the Langerhans cell component early after treatment, which returned to baseline pre-cycle 2. A smaller sustained increase in the natural killer cells (NK) and T CD8 TIL cell population in tissue, and in blood NK was also seen.

The change in tumour population in the microenvironment related non-significantly to change in skin disease of the patient. Patients who did not show a decrease in the microenvironment tumour population progressed during treatment.

PD-1 was most expressed on T-cells. Treatment resulted in an increase in PD-1 expression on the tumour population, as well as the T CD4 TIL, which correlated with CD25 and HLA-DR expression.

Conclusion
This study helps us to understand how blockade of a key immune signalling pathway can have multiple effects on the complex network of immune cells within the microenvironment of cancer.

P.16 **Developing new tests to measure measles immunity**

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In 2024, the UK Health Security Agency declared a major incident following a measles outbreak involving 347 confirmed cases. This outbreak highlights declining MMR vaccine uptake, now the lowest in a decade. Measles immunity is usually determined by IgG antibody quantification by ELISA. However, less is understood about T-cell mediated immunity, especially in infected, immunocompromised, or seronegative individuals. The scarcity of T-cell assays reflects complexities in the standardisation and scalability of cellular assays like ELISpots. Additionally, antibody and T-cell assays require the collection of venous blood, limiting the feasibility of large-scale or community screening.

In collaboration with ImmunoServ Ltd, researchers from Clinical Immunology Services are optimising a whole blood T-cell assay (Immuno-T™), which involves stimulating blood with measles peptides, and quantifying IFN-ɣ release by ELISA. This method removes leading limitations of traditional cellular assays such as ELISpots. Furthermore, capillary blood collection methods are being explored as less invasive and remote alternatives to venepuncture.

Measles antibody quantification from dry blood spots has shown 95% concordance with matched serum, demonstrating capillary blood as a promising alternative to venous blood. Immuno-T™ and ELISpots are currently undergoing optimisation to elicit T-cell responses, evaluating various panels of measles peptides.

This project addresses two major challenges in measles immunity monitoring: Developing standardisable and scalable T-cell assays and validating less invasive and remote blood sampling. Assessing humoral and cellular immunity simultaneously will provide a clearer picture of population-level protection, help inform infection risk in immune-vulnerable patients and help guide vaccine strategies and public health responses to outbreak.

P.17 **Optimising CAR T-Cell Therapy: Pulsatile Optogenetic Activation to Enhance Durability and Efficacy Against Tumours**

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Chimeric Antigen Receptor (CAR) T-cell therapy, involving the extraction of patient T-cells and arming them with receptors designed to target tumour specific antigens, has revolutionised the treatment of haematological malignancies in recent years. Despite the notable successes, CAR T-cell therapy only confers a durable response in 30-40 % of patients and exhibits a limited efficacy against solid tumours. Rapid exhaustion of CAR T-cells in response to persistent antigen stimulation is a known contributor to this limitation.

Our work aims to explore the use optically controllable pulsatile activation, thereby reducing the total CAR T-cell stimulation time, as a method of reducing cell exhaustion. Primary CD4+ T-cells are transduced with a photoactivatable anti-CD19 CAR construct. These OptoCAR T-cells are then co-cultured with CD19+ Raji B-cells under varying pulsatile sequences of light before conventional flow cytometry and/or spectral cytometry was employed to explore their phenotype. Varied pulsatile signalling regimens led to altered expression of both co-inhibitory (e.g. PD-1) and co-stimulatory (e.g. ICOS) receptors on the surface of OptoCAR T-cells.

Recently, Th9 cells have been observed to have a more robust effectiveness against tumours, possessing an enhanced longevity. We observed that pulsatile activation of OptoCAR T-cells was able to induce a Th9 phenotype (IL-9+), in the absence of TGF-β and IL-4 cytokine culture. Our data suggests that pulsatile activation warrants further investigation as a novel method for enhanced polarization of conventional CAR T-cells to a potentially more durable and effective therapeutic modality in the form of Th9 CAR T-cells.