Metabolic Health PGR Conference 2025

Abstract Booklet

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10-minute talks

Katherine Hampton: “In response to infection plasminogen activator inhibitor 1 (PAI-1) induces long-chain fatty acid availability to allow metabolic reprogramming and expansion of immune cells”

Katherine Hampton[1], Alyssa Polski-Delve[1], Rebecca S Maynard[1], Gwénaëlle Le Gall[1], Domonic Fowler-Shorten[1], Matthew Markham[1], Tabitha Bartlett[1], Nilda Iker[1], Charlotte Hellmich[1,3] , Naiara Beraza[2], Kristian M Bowles[1,3] and Stuart A Rushworth, PhD[1]

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During infection, hematopoietic stem and progenitor cells (HSPCs) switch to oxidative phosphorylation over glycolysis. This metabolic switch increases energy production in the form of ATP and allows the HSPC pool to expand and differentiate into downstream immune cells to produce an effective immune response to the infection. Fatty acid (FA) uptake and metabolism plays a central role in providing sufficient energy for HSPC expansion. However, the mechanisms that drive these changes are unknown. This study investigates the role of the liver in regulating FA availability for HSPC expansion. Liquid Chromatography-Mass Spectrometry revealed significant increases in circulating long-chain fatty acids (LCFAs) in mice in response to infection (lipopolysaccharide (LPS) and *Salmonella Typhimurium* models). Since the liver is the master regulator of serum FA availability, bulk RNAseq on livers isolated from LPS treated mice was performed and revealed down-regulation of several key genes involved in FA metabolism. Using primary hepatocytes, we show that this was caused by circulating cytokines found in the serum in response to infection. A cytokine array of serum from LPS treated mice revealed significant upregulation of several cytokines and chemokines. Of these only Plasminogen activator inhibitor-1 (PAI-1) significantly decreased LCFA uptake, transport and metabolism in primary hepatocytes. Furthermore, mice treated with recombinant PAI-1 showed significantly increased levels of circulating LCFAs and a decrease in liver lipid and FA metabolism genes. Pharmacological inhibition of PAI-1 prevented this downregulation of liver FA metabolism in response to LPS.

The majority of PAI-1 (up to 90%) is stored in platelets. We demonstrate that platelets are rapidly activated to release their stored PAI-1 in response to infection. Depleting platelets from mice prior to LPS treatment also significantly reduced the downregulation of liver FA metabolism, reducing circulating LCFAs and affecting HSPC expansion.

These findings demonstrate that circulating levels of LCFAs increase in response to infection to support immune cell expansion. This increase in LCFAs is at least partly driven by PAI-1 released from platelets which downregulates liver FA uptake and metabolism, allowing LCFAs to remain in circulation.

Mehmethan Aris: "The function and regulatory interactions of mitochondrial Uncoupling Proteins 1 and 2 in metabolic health"

Mehmethan Aris, Riccardo Cavalieri, Danielle Copeman, Margeoux A. S. Dela Rosa, Hannah R. Staggs, Steve Harborne, Paul G. Crichton

Uncoupling protein 1 (UCP1) occurs in specialised brown adipose tissue (BAT), where it leaks protons across the mitochondrial inner membrane to uncouple oxidative phosphorylation and release nutrient energy as heat. This thermogenic activity helps mammals survive cold temperatures. The protein is inhibited by purine nucleotides (e.g. ATP and GTP) but activated by fatty acids in response to the cold, and if targeted for activation in humans, has the potential to increase calorie expenditure to combat obesity and type 2 diabetes. The UCP1-homologue, UCP2 (59% sequence similarity), occurs in various other tissues and has pathophysiological roles in cancer and diabetes, making it a therapeutic target. The function and ligand binding properties of UCP2 are unclear, which likely relates to the challenges in producing and studying these types of unstable membrane proteins. Though emerging evidence suggests UCP2 may be distinct from UCP1 with a different biochemical function unrelated to proton leak.

Our studies aim to recombinantly produce UCP2 and assess the ligand-binding and functional properties of the protein, for comparison to UCP1. To provide robust reference data, UCP1 isoforms (human, mouse and ovine), produced in yeast and purified using established systems, were characterised by ligand-induced thermostability shift analysis and liposome activity assays, with ovine UCP1 from native sources included as a control. Surprisingly, our results indicate that human UCP1 interacts with nucleotides differently compared to other mammalian isoforms. Whilst adenosine nucleotides stabilise mouse and ovine UCP1 to a lesser degree than guanosine nucleotides, consistent with a known lower affinity, in contrast, human UCP1 is stabilised more by adenosine nucleotides over guanosine nucleotides. These findings suggest species-specific differences in UCP1 nucleotide regulation. Furthermore, our results indicate that UCP1, irrespective of the isoform, also interacts with pyrimidine nucleotides to varying degrees (e.g. TTP and CTP), aligning with recent literature findings. In parallel work, our trials to express human UCP2 in insect and mammalian cells show that versions of this protein with added purification tags can be successfully generated in an intact form. In future studies, UCP2 will be purified to elucidate its biochemical function in comparison to UCP1, utilising our methodologies developed for UCP1.

Lina Maarouf: “Metformin; The Antidiabetic as An Antibacterial Drug”

Lina Maarouf, Mark Webber, Benjamin A. Evans

Metformin, a widely used antidiabetic drug prescribed to more than 120 million patients globally annually, has shown some antibacterial activity against different bacterial genera. Few studies have investigated this; however, its mechanism of action on bacteria is still unclear. This study aimed to understand more about metformin’s effect on bacteria at a phenotypic or molecular level. The impact of different concentrations of metformin was tested on the growth of a selected Gram-negative species (Pseudomonas aeruginosa ATCC 27853) and a Gram-positive one (Staphylococcus aureus NCTC 6571). Impacts on biofilm formation, protease production, motility and efflux were also investigated. In the presence and absence of the drug, the total RNA of two species of P. aeruginosa and S. aureus were extracted and sequenced for transcriptomic analysis. In addition, a transposon library of the P. aeruginosa strain has been constructed, exposed to different concentrations of metformin and sequenced to reveal the difference between the various conditions and control. Finally, the effect of metformin on the respiratory chain of both strains was investigated using Biolog Mito plates and compared with other known respiratory chain inhibitors.

Stephanie Smith: "The prognostic value of Prostate Urine Risk: Decade-long clinical trajectories after urinary biomarker testing in the outpatient clinic"

Stephanie F. Smith[1,2], Kate Manley[1,2], Sergio Llaneza-Lago[1], Jeremy Clark[1], Rob Mills[2], Colin Cooper[1], Daniel Brewer[1]

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Introduction
Our group developed a urinary biomarker called Prostate Urine Risk (PUR), which is based upon a 38-gene expression readout of prostatic extracellular vesicle RNA. Previous work using prostatectomy specimens suggested PUR correlates to the volume of Gleason 4 disease. In this study, we aimed to evaluate the long-term clinical outcomes over a ten year follow-up period in a cohort who underwent PUR testing during outpatient clinic evaluation.

Methods
Participants gave informed written consent and provided urine samples for PUR testing following digital rectal examination in the clinic between 2012 and 2014. Extracellular vesicles were harvested, RNA extracted and NanoString expression analysis performed. Clinical follow-up data was collected using electronic hospital systems as part of the patient's standard clinical care. Ethical approval for this work was granted by the HRA (EC reference 12/EE/0058, IRAS project ID 96199).

Results
469 patients were recruited, of which 246 had a histological diagnosis of prostate cancer. The PUR result was significantly associated with prostate cancer specific survival. Among men with an initial benign biopsy (n=127), a high PUR-4 result (cut-off 0.174) was predictive of a future diagnosis of prostate cancer up to 12 years later (log-rank, $p<0.05$). In an active surveillance cohort, PUR was predictive of disease progression (log-rank, $p=0.0094$).

Conclusions
We provide proof of concept that PUR can predict clinically relevant outcomes in a cohort with a long period of follow-up. We are currently further evaluating the utility of PUR on urine samples collected using a home kit in a large multi-centre study.

Amy Harden: "Investigating the RNA structurome of Ewing sarcoma"

Amy M. Harden[1], Yiliang Ding[2], Darrell Green[1]

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The commonest driver mutation in Ewing sarcoma (EWS) is an in-frame *EWSR1::FLI1* gene fusion generating a chimeric oncogenic transcription factor. Despite relatively few other major mutations, EWS is clinically heterogenous. The mechanisms underlying this heterogeneity are unknown, but might be explained by the fact that EWS cells display heterogenous levels of oncoprotein, in phenotypic states termed EWSR1::FLI1^{high} and EWSR1::FLI1^{low}. EWSR1::FLI1^{high} is linked to a highly proliferative tumour phenotype, while EWSR1::FLI1^{low} has been associated with a more migratory/metastatic phenotype. Mechanisms influencing *EWSR1::FLI1* RNA abundance and oncoprotein output are unknown. The study of RNA beyond its' sequence and 'messenger' role is a novel but rapidly growing field. Cellular RNA are in fact highly dynamic and can take on a variety of sequence-independent three-dimensional structural conformations that play key gene regulatory roles. For example, riboswitches are RNA structural motifs that change conformation upon ligand binding to alter the accessibility of ribosome binding sites, therefore acting in a post-transcriptional regulatory manner. In the current study, we are characterising the RNA structurome in EWS, specifically the structure of the *EWSR1::FLI1* fusion transcript, to identify potential mechanisms of plastic EWSR1::FLI1$^{high \rightarrow low}$ and EWSR1::FLI1$^{low \rightarrow high}$ states. We are employing 2′-hydroxyl acylation and primer extension (SHAPE); this involves chemically probing RNA which acylates the 2'-OH of unpaired ribonucleotides causing the reverse transcriptase enzyme to 'drop off' the template strand during library preparation. The reactive bases can be identified at the bioinformatics step by comparing SHAPE-treated samples with DMSO controls to produce *in vivo* RNA structures. These structures can be compared visually and statistically to highlight structural changes and impact on mRNA abundance and oncoprotein output. The RNA structure of *EWSR1::FLI1* has been determined, and the next set of experiments will be to describe the role of specific motifs, such as G-quadruplexes and i-motifs, on post-transcriptional regulation and oncoprotein expression.

Seshadhri Subramanian: "In-silico functional profiles of anaerobic bacteria in prostate cancer"

Seshadhri Subramanian, Abraham Gihawi, Rachel Hurst, Colin Cooper, Daniel Brewer

Introduction

Prostate cancer is among the most common cancers globally (1 in 8 men in the UK will be diagnosed in their lifetime). Major risk factors include age, ethnicity, and family history. Microbial infections contribute to many cancers, and the prostate microbiome influences prostate cancer. Identifying the Anaerobic Bacterial Biomarker Set (ABBS) bacteria—Fenollaria, Ezakiella, Peptoniphilus, Anaerococcus, Porphyromonas, and Fusobacterium—may help predict aggressive prostate cancer at diagnosis. ABBS-positive samples are linked to more aggressive disease, and the mechanisms of actions of these bacteria are important to investigate.

Material and method

Whole genome sequencing data from 650 tumour samples in the Pan-Prostate Cancer Group (PPCG) were analysed for microbial content. Unmapped reads were quality-trimmed, human-depleted, and classified using Kraken. The metabolic profiles of ABBS-positive and negative samples were assessed using HUMAnN (v3.7). Gene families and pathways were analysed with Maaslin2 (v1.12.0) in R (v4.2.0).

Result and discussion

ABBS-positive tumours had 888 significantly enriched gene families and 336 metabolic pathways, including genes for tRNA synthetases for all 20 amino acids and methionine synthesis/salvage pathways. This was significantly higher than the 75 gene families and 17 pathways found in ABBS-negative tumours. Key ABBS-associated pathways include methionine salvage/synthesis and tRNA charging. Cancer cells rely heavily on external methionine (Hoffman effect) and arrest in the S/G2 cell cycle phase under methionine restriction. Methionine metabolism may be a core feature of cancer development. tRNA charging, facilitated by aminoacyl-tRNA synthetases (ARSs), enables peptide bond formation. Aberrant ARS expression is linked to the transformation from benign to malignant cells, with ARS-encoding genes exhibiting expression profiles similar to Cancer-Associated Genes (CAGs) in 10 cancers.

Conclusion

These findings suggest microbial metabolism may contribute to prostate cancer progression when ABBS bacteria are present. Future work will look at mutational profiles in the PPCG that are associated with ABBS positive tumours, identifying additional bacteria with a similar metabolic profile and investigating the relationship of enzymes and pathways affected in ABBS positive tumours with long term survival in ABBS positive patients.

Bernadette Breeze: “Exploring Female Alzheimer’s disease vulnerability in mouse models”

Bernadette Breeze, David Vauzour, Tom Wileman, Michael Muller, Matthew Pontifex

Alzheimer’s Disease (AD), responsible for 62% of all dementia cases, is a progressive neurodegenerative condition that leads to cognitive dysfunction. The prevalence of AD is significantly greater in women, attributable in part to greater lifespan and cardiovascular disease susceptibility. Given the multifactorial nature of Alzheimer’s disease, it is plausible that female-specific mechanisms exist. Menopause has been suggested to be a contributing factor in AD and dementia development. Indeed, women who develop menopause at an earlier age, whether through surgical or natural menopause, display greater impairment of cognition and increased overall dementia incidence. The underlying mechanisms responsible for these cognitive deficits and subsequent dementia risk are yet to be fully elucidated.

Favoured rodent models of AD tend to develop AD pathology at a young age (approximately 3-6 months old) and are therefore more representative of early-onset AD which accounts for approximately 5% of all AD cases. In addition, these models do not necessarily replicate all the pathological hallmarks of the disease. In contrast, the Atg16LΔWD mouse model (utilised in this study) in which the WD domain of endocytosis machinery Atg16L is deleted, develops spontaneous late-onset AD, recreating all the key pathologies of AD (including amyloid-beta deposition, microglia activation, and tau hyperphosphorylation)

Fifty mice were utilised, 25 with WD domain deletion, and 25 without (control). At 8 months of age, half of the animals had menopause chemically induced. Behavioural tests conducted at 12 months of age revealed a large, significant genotype-mediated increase in anxiety as assessed by the open field task. RNA sequencing analysis highlighted both genotype and menopause-related changes in gene expression profiles within the brain, with the hippocampus particularly influenced. Results from pathway analysis indicate dysregulation in mitochondrial function and synaptic plasticity. Immunohistochemistry shows that menopause exacerbates the AD phenotype by increasing amyloid-beta depositions, hyperphosphorylation of tau, and increased activation of microglia.

Anna Russell: "Molecular mechanisms of anaerobic bacterial infection on prostate cancer development and progression"

Ferroptosis is a distinct form of regulated cell death, characterised by iron-dependent lipid peroxidation. We recently reported that specific bacterial infections are associated with enhanced prostate cancer aggression and more rapid progression, notably, that of *Fenollaria sporofastidiosus and Anaerococcus prevotii.* In this study, we aimed to investigate the molecular mechanisms underlying this association, focusing on how *F. sporofastidiosus* and *A. prevotii* infection may regulate ferroptosis in prostate cells. Microarray analysis revealed a significant upregulation of the gene *SLC7A11* in prostate cells infected with *F. sporofastidiosus and A. prevotii*, with *SLC7A11* being the most highly upregulated gene in prostate cells infected with *F. sporofastidiosus*. Given that *SLC7A11* plays a critical role in regulating ferroptosis, we hypothesised that *F. sporofastidiosus* infection inhibits ferroptosis by upregulating ferroptosis inhibitors such as *SLC7A11* and *GPX4*. This hypothesis was further tested through western blot analysis, cell viability assays and lipid peroxidation assays. Our results suggest that *F. sporofastidiosus and A. prevotii* infection inhibit ferroptosis in prostate cells, offering new insights into the bacterial modulation of cell death pathways and their potential role in prostate cancer progression.

Tim Brendler-Spaeth: "Predicting Aggressive Prostate Cancer with Multi-Omics and Machine-Learning"

Prostate cancer (PCa) is the second most common cancer in men worldwide with a 1 in 8 chance of being diagnosed in their lifetime. PCa exhibits considerable heterogeneity; some patients demonstrate aggressive disease, characterised by rapid progression, while other cases are indolent and remain harmless for many years. Hence, accurate prognostication at time of diagnosis is crucial for guiding treatment decisions.

Disease stratification is a significant clinical challenge due to PCa's heterogeneity. Current methods often fall short in accurately differentiating between aggressive and indolent forms of PCa at time of diagnosis. Inaccurate prognoses can lead to overtreatment with many patients undergoing radical treatments with life-changing side effects that may be unnecessary. Prognostic biomarker tests currently available often utilise only a single 'omic. Research previously conducted by our group and elsewhere shows that improved prognostic power may be gained via a multi-omic approach.

Our group uses multi-omic datasets, consisting of genomic, methylomic, and transcriptomic data from PCa patients. With their extensive clinical information, these data provide a comprehensive resource to identify multi-omic biomarkers for predicting aggressive PCa. We have integrated multiomic data together with several machine learning algorithms with the aim of producing a robust predictor of disease progression. In addition, we have also determined associations between the different 'omic layers to gain a better understanding of disease aetiology. These investigations were conducted at a "global", genome wide as well as individual gene level.

Emma Bull: "Single-cell analysis reveals circulating tumor cells promote the transcription of dark genome regions"

Emma C. Bull [1], Archana Singh [1], Sergio Llaneza-Lago [1], Lee Jeys [2], Vaiyapuri Sumathi [2], Darrell Green [1]

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In a normal somatic cell it is estimated that ~80% of the human genome is transcribed into RNA molecules but only ~2% are protein coding. Cancer cells can hijack these coding/non-coding genes and transform cells into deadly malignancies. Defining which cancer cells hijack which genes has been the backbone of contemporary precision oncology, disease subtyping, and targeted therapy development. Much of this knowledge has been generated through the analysis of donated primary tumors; however, metastasis is the leading cause of cancer related death and is thought to be an independent biological process to tumor development and probably requires different sets of gene networks and cell phenotypes. Metastasis is a distinct disease component requiring specific (sub)clones to undergo complex phenotypic plasticity not required since embryogenesis; for example, detachment, invasion, migration. A significant fraction of the genome is not needed by normal cells but this 'dark matter' of the genome could be transcribed by metastatic cells to achieve systemic disease. The human genome harbours remnants of our evolutionary history and includes transposable elements, pseudogenes and viral sequences.
Whilst there are mechanisms in place to safeguard against pathogenic gene expression (e.g. piRNAs), not all sequences are inaccessible. Some sequences are transiently expressed. One example is the human endogenous retrovirus-W (HERV-W) derived gene required for placental morphogenesis. Under the opportune conditions including amplifications and gene rearrangements generating novel oncogenic transcription factors, metastatic cells could engineer new RNA molecules and gene networks not observed in normal or even tumor cells.
Here, we took an unbiased approach and performed single-cell RNA sequencing (scRNA-seq) in 'metastatic' osteosarcoma and Ewing sarcoma patient derived circulating tumor cells (CTCs) and discovered a completely new cohort of unannotated RNAs. Experimental work to determine the clinical significance of these RNAs is underway, using gain- and loss-of-function mutants and orthotopic xenograft mouse models.

3-minute talks

Calista Tink: “Investigating Prostate Cancer Molecular Subtypes at the Single-Cell Level”

Calista Tink, Sergio Llaneza-Lago, Colin Cooper, Harveer Dev, Daniel Brewer

In the UK, one in eight men will face a prostate cancer diagnosis in their lifetime. Prostate cancer is a heterogeneous disease that exhibits diverse clinical behaviour, ranging from indolent to highly aggressive phenotypes. This variability hinders the effective clinical management of prostate cancer due to our current inability to accurately stage and predict disease course at the time of diagnosis.

Molecular classification is an approach that uses the underlying biology of tumours to categorise them into clinically relevant subtypes. Molecular subtypes can capture the molecular heterogeneity of tumours better than the singular biomarker tests currently used in the clinic, potentially leading to more accurate prognostic predictions. Luca et al., (2017) proposed that individual prostate cancer samples can contain more than one contributing lineage. The authors applied Bayesian unsupervised Latent Process Decomposition (LPD), which accounts for the presence of multiple lineages, to bulk sequencing data and identified a novel aggressive prostate cancer subtype. However, bulk sequencing averages gene expression across a sample, which could omit rare cell populations and key molecular drivers. Single-cell sequencing offers a new perspective that could better capture the heterogeneity within a sample by clustering individual cells together based on their gene expression.

Initial work has entailed the preprocessing of 15,887 cells from a 10-patient single-cell RNA sequencing dataset. Preliminary results identify the known prostate cancer marker genes MSMB and KLK3 among the top 20 genes with the most variable expression in the dataset. Efforts to apply the Luca et al., LPD model to our samples to assess the presence of the molecular signatures are ongoing. In the future, I will apply soft-clustering approaches to investigate and characterisation novel molecular subtypes in single-cell data. Moreover, I will utilise a single-cell atlas with over 100,000 cells to expand the dataset and achieve a more thorough analysis.

Alyssa Polski-Delve: "Ageing alters changes to liver fatty acid metabolism in response to infection"

During infection, haematopoietic stem and progenitor cells (HSPCs) have increased energy demands to facilitate expansion and differentiation. These demands are met by an upregulation of fatty acid (FA) uptake, metabolism, and use in oxidative phosphorylation. Ageing is a well-known risk factor for serious infections, where chronic low-grade inflammation and Immunosenescence impact HSPC and overall immune function. This study examines alterations in FA metabolism during infection in aged mice and the impact this has on immune function.

Aged mice (18-24 month) were administered lipopolysaccharide (LPS) for 2 and 6 hours. Liquid-chromatography mass spectrometry performed on serum indicated that long-chain FAs do not increase in response to LPS. In young mice, serum long-chain FAs are known to increase during infection.

The liver is the principal site of lipid metabolism. In young mice LPS induces down-regulation of FA metabolism in the liver. Here we show, using bulk RNA-seq of aged LPS mouse liver tissue and KEGG pathway analysis, that FA metabolic processes are not downregulated during infection, unlike young. Hepatic lipid metabolism-related gene expression in aged LPS mice revealed small, early decreases. However, expression was fully restored by 6 hours post infection, suggesting that the response is not sustained. Hepatocytes in vitro treated with serum from aged mice at 2 and 6 hours post infection indicate a similar pattern of gene expression.

We hypothesise that aged mice are not able to sustain the hepatic FA metabolic response to LPS, causing a lack of available FAs for HSC uptake, and subsequent inability to clear infection.

Peter MacCallum: “Investigating the effect of short-chain fatty acids on the multiple myeloma environment”

Peter MacCallum, Dominic Fowler-Shorten, Helena Pardo-Casales, David Vauzour Stuart Rushworth, Nicole Horwood

Multiple myeloma (MM) is a haematological malignancy characterized by uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM), spleen, and peripheral blood. A hallmark of MM is excessive Immunoglobulin G production, often leading to renal disease and a weakened immune response. MM is preceded by an asymptomatic stage known as monoclonal gammopathy of undetermined significance (MGUS). Despite significant advances in immunotherapy, MM remains incurable.

As MM primarily affects the aging population (35% of patients diagnosed are over 75), patients often exhibit altered gut microbiota, including reduced short-chain fatty acid (SCFA)-producing species. SCFAs, including butyrate, propionate, and acetate, are gut-derived metabolites from dietary fibre fermentation with anti-tumour properties in MM studies. MM cells proliferate in the BM, supported by other cell types such as macrophages and adipocytes. This study hypothesises that culturing MM cells with SCFAs will promote anti-tumour effects in co-culture models by enhancing immune activation and suppressing malignant proliferation.

The direct addition of SCFAs to 5TGM1 (MM cell line) did not affect proliferation or the mRNA expression of inflammatory cytokines and metabolic regulators. To investigate the involvement of other cell types from the bone marrow, co-cultures of murine bone marrow-derived macrophages with 5TGM1 and SCFAs were established. Using a combination of viability assays and qPCR, it was shown that the presence of macrophages and SCFA reduced 5TGM1 viability and that IL-6 and IFN□ mRNA expression by the macrophages was increased.
These findings to date suggest that the effect of SCFA on MM cell viability is not direct and requires the presence of other cells associated with the BM. Further studies will be undertaken to determine how the macrophages are able to reduce 5TGM1 viability and whether other cells of the BM are also able to influence 5TGM1 cells in the presence of SCFA.

Dominic Fowler-Shorten: "Age-Associated Macrophage Polarization to an M2 Phenotype Drives Myeloma Proliferation in the Aged Bone Marrow by Loss of Tumor-Associated Phagocytosis"

Dominic Fowler-Shorten, MRes, BSc[1], Charlotte Hellmich, PhD, BSc, MBBChir[1,2], Diego Pereira-Martins[3,4], Annalisa Altera, PhD[1], Rebecca Maynard, MSc[1], Katherine Hampton, MSc, BSc[1], Matthew Markham, PhD[1], Alyssa Polski-Delve, MSc, BSc[1], Kristian Bowles, MBBS, PhD[1,2] and Stuart A Rushworth, PhD[1]*

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Background:
Multiple myeloma (MM) hijacks cellular interactions in the bone marrow microenvironment (BMM) to promote its survival and progression (1,2). M2-like macrophages reportedly exhibit a pro-tumoral phenotype that supports immune BM remodelling in MM (3,4). Further, the senescence associated secretory phenotype (SASP) promotes a pro-tumoral BMM with age (5,6). Thus, age-related BMM changes may drive MM development.

Aims:
Using in vitro and in vivo modelling, we aim to investigate if ageing drives changes in BM macrophage phenotype and function that may promote MM.

Methods:
5TGM1 myeloma cells were established in the aging KaLwRij mouse model (7). We and others have shown that 5TGM1 cells do not engraft in young C57BL/6 mice but can engraft in obesity induced C57BL/6 mice (8). To determine if aging C57BL/6 BM is permissive to 5TGM1 cells, we injected the cells intravenously into young (3-4 months), middle-aged (12-14 months), and aged (18-22 months) mice. After 30 days, BM and spleen were harvested for flow cytometry. BM from non-injected young and aged C57BL/6 mice were analysed by flow cytometry for macrophage populations and phagocytosis (pHrodoTM assay). To explore the effect of M1 and M2 macrophages on 5TGM1 viability, bone marrow-derived macrophages were cultured and stimulated to M1 or M2-polarisation state. 5TGM1 cells were co-cultured with M1 and M2 macrophages for 48hr to assess 5TGM1 cell viability. Using publicly available 10x scRNA-seq data, we analysed the metabolic profile of M1 and M2 macrophages in myeloma (n=24) vs healthy patients (n=12) by differential expression analysis. Lastly, aged C57BL/6 and KaLwRij mice were both engrafted with 5TGM1 and macrophages isolated to assess expression of NAD+ biosynthesis genes by qPCR.

Results:
We show that 5TGM1 cells engrafted in all aged mice (7-34% BM GFP+, 3-26% splenic GFP+), while only 2/9 middle-aged mice had minimal engraftment (<2% BM GFP+) and no young mice engrafted. To investigate the underlying mechanism

permitting 5TGM1 engraftment in the aged C57BL/6 BMM, flow cytometry identified an elevated total macrophage count in aged mice compared to young, concomitant with an overall elevated M2/M1 ratio in the aged mice. Additionally, 5TGM1 viability was reduced in M1 co-culture vs co-culture with M2 or 5TGM1 cells alone, which correlated with an impaired phagocytic capacity in the aged BM vs young. Preliminary scRNA-seq analysis identified upregulation of M2 but not M1 gene sets in myeloma patients from four independent datasets vs healthy patients, together with upregulated expression of nicotinamide phosphoribosyltransferase (NAMPT), though its expression was heterogenous across the macrophage cluster. Finally, we verify that NAMPT is upregulated alongside other NAD+ biosynthesis genes in macrophages from aged C57BL/6 and KaLwRij engrafted mice.

Summary/Conclusion:
Here, we show that aged C57BL/6 mice are permissive of 5TGM1 cells. We show that this may in part be mediated by an elevated M2/M1 ratio in the aged BMM and demonstrate that macrophages in the aged BM have a reduced phagocytic capacity. Taken together, these data suggest that a loss of M1-like phenotype or a shift toward the M2-like phenotype in the aged BMM may confer a permissive, anti-inflammatory state for MM development. We also identify NAMPT as a potential metabolic target in myeloma-associated (M2) macrophages – future work will assess the effect of NAMPT inhibition on macrophage phenotype and function using our existing models.

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Kirsty Soanes: "Investigating the causes and consequences of PAX3:FOXO1 RNA structural plasticity"

Rhabdomyosarcoma (RMS) is a soft tissue sarcoma that is thought to arise from abnormally terminally differentiated skeletal muscle progenitors. Some RMS subtypes are driven by gene rearrangement between PAX3 or less commonly PAX7 but in both cases to FOXO1. Expression of the PAX3/7::FOXO1 fusion is associated with a poorer disease prognosis when compared to RMS cases lacking the fusion. Fusion positive RMS (FPRMS) mainly occurs in children <10 y. Five-year overall survival is ~50% in cases with localised disease at diagnosis, dropping to <30% in advanced disease. The encoded fusion oncoproteins function as pioneer transcription factors, activating myogenic super-enhancers and establishing autoregulatory loops to drive the oncogenic transcriptional programme. Much FPRMS research has focused on the roles of the oncogenic transcription factors and their DNA-binding. Less research has examined the messenger RNA (mRNA) transcript for PAX3/7::FOXO1 and how clinical modulation of the transcript rather than the protein might be a better target for treatment development. Cellular RNAs including those from PAX3/7::FOXO1 are diverse with respect to their alternative processing and secondary and tertiary structures. Sequence independent RNA structure ,and mRNA structure isoforms, constitute a new layer of gene regulation. RNA-binding proteins (RBPs) can modulate RNA structure and access to the ribosome binding sites (and possibly microRNA recognition elements in the 3' UTR); therefore, participate in post-transcriptional regulation, protein levels and cell identity. cDNA libraries have been generated from the chemically probed RNA of RH4 human FPRMS cells to experimentally reveal the in vivo structure of PAX3::FOXO1 mRNA and were sequenced using Oxford Nanopore. The work will be repeated to check the robustness and repeatability of the methods, before progressing the project where the RH4 RNA structurome will be modified to investigate the causes and consequences of RNA structural dysregulation in FPRMS; with an intent to identify opportunities for clinical intervention.

Ria Rigana: "Targeting Triple-Negative Breast Cancer: Dual Action of Carfilzomib and Benzyl Isothiocyanate"

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, accounting for a significant proportion of cases in the UK and worldwide, with an estimated incidence rate of 11.7% according to the World Health Organization (WHO). Unfortunately, existing treatment options often lead to off-target effects, resulting in adverse events and resistance to treatment. Hence, there is an urgent need for alternative treatments aimed at minimizing adverse events and enhancing prognosis. Carfilzomib shows significant potential in targeting tumors with high proteasomal activity, particularly in aggressive subtypes like TNBC, by inhibiting proteasome function and disrupting key survival pathways. Additionally, isothiocyanates (ITCs), derived from glucosinolates found in cruciferous vegetables, have lately captured attention due to their anticancer activities. In this study, the interactions between ITCs and carfilzomib are being explored for understanding the mechanism of action and their potential in triple-negative breast cancer. Results demonstrated that the combination of carfilzomib (CFZ) and benzyl isothiocyanate (BITC) exhibited synergistic effects in MDA-MB-231 cells, with selective activity when compared to MCF10A and MCF7 cells. Flow cytometry revealed an antagonistic effect on apoptosis and cell cycle. Reactive oxygen species (ROS) levels were elevated with single BITC treatment and in the combined CFZ+BITC treatment, though no significant enhancement was observed in the combined treatment. Furthermore, NOX-4 was upregulated in both single and combined treatments, with the upregulation further amplified in the combined therapy. The LC3II/LC3I ratio, indicative of autophagosome formation, was elevated in both single BITC and combined treatments, suggesting activation of autophagy. Additionally, p62 accumulated in single treatments, particularly with carfilzomib, but this accumulation was reduced when both drugs were combined. No significant changes were observed in Beclin 1 levels, suggesting a selective modulation of autophagy mechanisms. These findings suggest a complex interaction between BITC and carfilzomib, with potential implications for therapeutic strategies in TNBC.

Helena Pardo: "The gut-muscle axis: the role of gut microbiome in skeletal muscle and ageing"

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Skeletal muscle mass, strength and regenerative capacity declines with age. This condition is known as sarcopenia. Many of these measures show a greater decline in females around the time of menopause, when oestrogen levels decrease. There is no current cure for sarcopenia.

Gut microbiome's diversity can get altered due to ageing and ageing conditions such as menopause. Dietary intervention has been proposed as a potential modulator of sarcopenia by directly influencing bacterial diversity and bacterial metabolites production. In addition, the gut microbiota also has been seen to modulate circulating oestrogen levels so, we propose a triple gut-muscle-oestrogen axis to modulate muscle wasting during ageing.

Two separate in vivo experiments were done to study the impact of dietary interventions and menopause in gut microbiome and skeletal muscle. To study the effect of high fibre diets, 10 weeks old C57BL/6 mice were fed with either a standard diet or high-fibre diet and sacrificed at 20 weeks. To assess the effect of menopause, 8 months old C57BL/6 female mice were injected with 4-vinylcyclohexene diepoxide (VCD) – a compound that gradually depletes the ovarian follicles, thus mimicking human menopause.

Caecal samples, gut and skeletal muscle were extracted. Histology was employed to assess skeletal muscle fibre quantity and type, and gene expression was assessed by RT-qPCR. Gut microbiota DNA was extracted from caecal samples and bioinformatically analysed via 16S rRNA sequencing. SCFA quantity in caecal samples was analysed by NMR.

High fibre dietary intervention showed a significant increase in alpha and beta diversity and metabolic profiling of the gut microbiome. It also showed a significant increase in muscle fibres size. Induction of menopause through VCD injections, had no impact in the composition or metabolic profiling of the gut microbiome. Skeletal muscle morphology and gene expression analyses are undergoing.

These results have been used to set up an ongoing experiment linking VCD injections and high fibre dietary intervention for the exploration of a possible gut-muscle-oestrogen axis.

Emily Sampson: "Using dietary isothiocyanates and Scutellaria compounds drive ferroptotic cell death in cancer"

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Phytochemicals and their metabolites are part of a plant's immune defence and can be used to treat various diseases due to their anti-microbial, anti-inflammatory and anti-cancer properties. For this reason, plant extracts, specifically Scutellaria barbata, are extensively used in Traditional Chinese Medicine. Many individual compounds from this plant, including flavonoids and diterpenoids, have gained attention for their anti-cancer properties. Clinical trials demonstrate effectiveness of an aqueous extract in treating breast cancer.

Another group of phytochemicals called isothiocyanates (ITCs), found in cruciferous vegetables, are associated with a variety of anti-cancer effects. These include modulation of phase I and II enzymes, cell cycle arrest, angiogenesis inhibition, and induction of multiple cell death pathways.

A recently discovered area of programmed cell death is ferroptosis, an iron dependent form, characterised by the accumulation of lipid peroxides. Central to ferroptosis is glutathione peroxidase 4 (GPX4), responsible for the inhibition of ferroptosis through prevention of lipid peroxide accumulation. Interestingly, research shows ITCs can also induce ferroptosis in multiple cancer types. S.barbata extracts have shown to induce ferroptosis in hepatocellular carcinoma cells.

Combinations of S.barbata extracts with sulforaphane, an ITC found in broccoli, has shown a synergistic effect. Importantly, S.barbata extracts downregulate GPX4 protein expression in two breast cancer cell lines. My ongoing study will include other key players in ferroptosis, including lipid peroxide induction and iron accumulation to verify the role of S.barbata compounds in ferroptosis.

Going forward, isolating single compounds from S.barbata identified via HPLC/MS analysis will provide exciting insights into the specific molecules responsible for inducing ferroptosis. They will also be used in combination with ITCs to uncover potential synergistic interactions, which are essential for developing cancer treatments with increased efficacy.

Combinations acting on ferroptosis could be highly promising for enhancing the effectiveness of conventional therapies, overcoming resistant tumours, and preventing tumours returning.

Posters

Matthew Neil: “Structural variants and the Acinetobacter baumannii genome”

Acinetobacter baumannii are a leading cause of nosocomial infections. They display a remarkable ability to thrive in various niches and acquire resistance to new antimicrobials. Studies in other bacterial species have shown that genome structural variants (SVs) are common and play a role in niche adaptation and antimicrobial resistance. However, there have been no large-scale studies into this phenomenon in *A. baumannii*. This study aims to investigate SVs in *A. baumannii* genomes using a large dataset of long-read genomes from both public databases and in-house isolates. Genomes will be represented as synteny blocks approximately the size of gene clusters to detect large SVs. Alterations in the positions of resistance and virulence factors with respect to the OriC will be used to infer possible phenotypic effects of each SV. This study will provide valuable insight into the genomics underlying *A. baumannii* adaption and resistance.

Ricardo Ackbersingh: “Enhancing Microbial Detection in Prostate Cancer: A Focus on African-Centric Data”

Supervisors: Prof. Daniel Brewer, Dr. Rachel Hurst, Dr. Abraham Gihawi, Prof. Colin Cooper, Dr Vanessa Hayes

Prostate cancer disproportionately affects individuals of African ancestry affecting 1 in 4 men, yet most research has focused on European populations, leaving critical gaps in understanding how the disease manifests in African individuals. Despite evidence suggesting that the microbiome may influence cancer progression, studies rarely incorporate African-centric data, limiting the relevance of current findings. My project addresses this disparity by improving microbial detection in prostate samples, ensuring that human DNA depletion methods e0ectively preserve microbial content. By refining metagenomic classification and leveraging African-centric sequencing datasets, I aim to identify microbial signatures linked to prostate cancer. This approach not only enhances our understanding of the microbiome’s role in prostate cancer but also ensures that African populations are represented in microbiome research. Future work will validate key bacterial species in urine and tissue samples, with the ultimate goal of identifying microbial biomarkers for prostate cancer risk stratification, providing insights that could inform more equitable diagnostic and treatment strategies.

Nilda Ilker: "Investigating the Metabolic Link between Acute Myeloid Leukaemia and Heart Failure"

Nilda Ilker[1], Ka Cheung[1], Terri Holmes[1], Tabitha Bartlett[1], Alyssa Polski-Delve[1], Annalisa Altera[1], Stuart A. Rushworth[1], James G.W. Smith[1]

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Leukaemia patients commonly develop cardiovascular disease and with many deaths occurring from heart failure later in life, despite being cleared from the cancer. This occurs at the highest rates in acute myeloid leukaemia (AML), compared to patients with other types of leukaemia. It is well established that chemotherapy is detrimental to heart health, but recent studies now indicate markers of heart failure (such as NTproBNP) as elevated in AML patients, prior to chemotherapy treatment. We hypothesise that cancer, and specifically AML, damages heart function and health, even before chemotherapy. To investigate this, I will isolate cardiomyocytes from AML-induced mice models and assess changes in gene and protein expression (qPCR/ RNA-seq/ western blot). In parallel, I will expose cardiomyocytes derived from human induced pluripotent stem cell (hiPSC-CMs) to AML patient serum to observe functional changes in vitro (contraction force analysis/ metabolic assays). Characterising how AML alters cardiomyocyte expression and function will provide further insights into the heart failure experienced by AML patients, improving our understanding in cardio-oncology. Furthermore, this work may identify key pathways and regulators with therapeutic potential and the development of improved treatments.

Athina Smallwood: "Unravelling interactions of genotoxic E. coli with human colonic epithelium and the microbiome under physiologically relevant conditions"

Athina Smallwood[1], Jean-Philippe Nougayrede[2], Nathalie Juge[1], Stephanie Schuller[1]

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Colorectal cancer (CRC) is the third most prevalent and second most deadly cancer worldwide. Notably, up to 10% of CRCs have been associated with *Escherichia coli* (*E. coli*) strains that produce the genotoxic metabolite colibactin. Colibactin induces DNA damage and interstrand cross-links in colonic epithelial cells, contributing to carcinogenesis. Recent studies have shown that colibactin expression is highest under microaerobic conditions. Furthermore, mucus depletion and inflammation enhance colibactin production, highlighting the role of the gut environment in regulating its expression. Colibactin also exhibits antibacterial properties, potentially serving an evolutionary function in interbacterial competition within the anaerobic gut ecosystem. To address the role of colibactin and the microbiome in CRC development, we will employ a physiologically relevant, microaerobic, mucus-producing human colonoid model that allows co-culture with oxygen-sensitive gut bacteria. This model will be used to investigate: (1) the influence of chemical cues and commensal bacteria on colibactin expression, (2) the interaction between genotoxic *E. coli* and the colonic epithelium, and (3) the impact of genotoxic *E. coli* on the gut microbiome. This project aims to advance CRC therapies targeting *E. coli* eradication and colibactin inhibition.

Ka Cheung: "The Role of the Extracellular Matrix in Cardiomyocyte Development"

Ka Cheung[1], Nilda Ilker[1], Terri Holmes[1], Linda Troeberg[1], James G.W. Smith[1]

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Proteoglycans are main components of the extracellular matrix (ECM) and are comprised of a protein core with attached disaccharide sugar chains, called glycoaminoglycans (GAGs). Recent studies have shown that heparan sulphate (HS) proteoglycans play key roles during cardiac development, modulating the proliferation and maturation of cardiomyocytes. HS proteoglycans are known to facilitate growth factor signaling, including FGF2 and Wnt signaling. This project will investigate the role of GAGs, specifically HS, in the differentiation and function of induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). HS GAG chains are formed through a series of enzymatic activity, including elongation by exostosin 2 (EXT2). In this project, wild-type (WT) and EXT2 knockout (KO) iPSC-CMs will be used as an in vitro model of HS loss during hiPSC-CM differentiation. Additionally, WT hiPSC-CMs will be exposed to chlorate treatment to inhibit HS synthesis following differentiation. Analysis will include developmental and cardiac-associated gene expression (by quantitative polymerase chain reaction) and functional proliferative assays (Ki67 and EdU assays). Insights into how GAGs can modulate the development and function of cardiomyocytes will allow for improved in vitro cardiac disease models and may identify new targets for the treatment of impaired cardiomyocyte function in heart failure.

Aaron White: “Acute Myeloid Leukaemia drives changes in megakaryocyte population in the lung”

Aaron White, Edyta Wojtowicz, Dominic Fowler-Shorten, Alyssa Polski-Delve, Katherine Hampton, Charlotte Hellmich and Stuart A Rushworth

Background
Acute Myeloid Leukaemia (AML) is an aggressive haematological malignancy, characterised by the uncontrollable proliferation of myeloid precursor cells in the bone marrow leading to impaired haematopoiesis. While AML primarily affects the bone marrow and peripheral blood, increasing evidence suggests the involvement of extramedullary sites, such as the lungs and liver, may also contribute to disease pathology and associated complications [1,2]. Recent research suggests megakaryocytes have a more broad function in inflammatory responses and that they can migrate to the lungs [3]. This raises the question of how these megakaryocytes are affected in an AML disease state, and how this is associated with pulmonary changes. This study investigates the changes in megakaryocytes populations and gene signatures in the lungs of mice with and without AML.
Methods
To interrogate alterations in megakaryocyte populations in AML disease we employed two syngeneic mouse models of AML, in which lineage negative cells transduced with MN1 or HoxA9/Meis1 oncogenes are engrafted into C57BL/6 mice. The lungs were perfused, isolated, and digested using collagenase II and DNase1. Isolated cells were stained for CD41 and Hoechst 33342 followed by analysis by flow cytometry.
Results
Results show changes in 2N and 4N lung megakaryocyte populations in mice engrafted with AML compared to controls. These results were verified using ImageStream analysis. Furthermore, lung megakaryocytes were bulk sorted using image cell sorting into 2N and 4N populations and qPCR analysis shows an increase in pro-inflammatory phenotype in AML compared to controls.
Conclusions
These results highlight the lungs, as well as megakaryocytes, as an area of further interest in AML research, and highlighting its potential role in disease pathology and the cause of pulmonary complications.

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Tabitha Bartlett: “Investigating the impact of age-related gut microbiome changes on acute myeloid leukaemia progression”

Acute myeloid leukaemia (AML) is a haematological malignancy characterised by the abnormal clonal expansion of immature myeloid progenitor cells, resulting in atypical and ineffective haematopoiesis. AML is a highly heterogeneous disease that predominantly affects elderly individuals, with a median age of diagnosis at 64. The disease commences with the clonal expansion of pre-malignant founder mutations in hematopoietic stem cells. Notably, as individuals age, clonal hematopoietic expansion becomes more prevalent, and founder mutations have been estimated to be identified in over 90% of individuals over 70 years old. However, only a small proportion of these individuals develop AML, suggesting extrinsic factors play a role in the disease progression. As our ageing population grows, there is an increasing need to elucidate the mechanisms involved in the transformation of pre-malignant clones to AML. The gut microbiome has been highlighted as critical player in shaping the immune system and maintaining homeostasis. During ageing, alterations in the gut microbiome composition are observed and emerging studies have revealed that the dysregulation of the gut microbiome plays a role in chronic low-grade inflammation. Here, we aim to elucidate whether age-related gut microbiome changes alter the delicate balance between the microbiota and the immune system, contributing to the progression of AML. To investigate this, we have developed an in vivo and in silico platform capable of performing taxonomic profiling and analysis of the gut microbiome during the onset of AML in ageing. This platform may enhance our understanding of the mechanisms involved in the progression of AML and could expose novel treatment strategies.

Molly-Kay Bailey: "The roles of microRNAs in cartilage circadian rhythms"

Osteoarthritis (OA) is a chronic joint disease characterised by gradual loss of articular cartilage (AC). Currently, OA affects 7.6% of the global population though clinical interventions are limited by OA's complex pathogenesis. The peripheral circadian clock and microRNAs are mediators of chondrocyte gene expression and, their deregulation is associated with AC degeneration. Therefore, an informed understanding of their intrinsic regulators may aid developments of novel therapeutics. This study has, for the first time, characterised inter-regulation between the chondrocyte molecular clock and miRNAs using an *in-vitro* and *in-vivo* AC model.

Circadian mechanisms pivot around core clock proteins oscillating in an autoregulatory feedback loop. Here, *CLOCK, RORα, PER2, CRY2* and *NPAS2* were targeted by hsa-miR-455-5p and hsa-miR-455-3p in 3'UTR luciferase reporter assays. Though, analysis of RNA-seq data from the AC of *miR-455* KO mice identified no significant changes in clock gene expression. Thus, exploring the role of miR-455 in determining phase and amplitude of core clock gene expression will be of value. Using a small-RNA sequencing model, 19.5% of miRNAs exhibited 24-hour oscillation in murine knee AC. Two-thirds of miRNAs peaked within the rodents rest-phase including miR-30b-5p, miR-30-3p, and miR-30c-2-3p of the miR-30 family. The 5p arms of the miR-30 family share a conserved 6-mer seed region and were differentially regulated by the clock, likely diversifying their biological function. The miR-17/92 cluster were co-regulated peaking at CT12 and, RT-qPCR determined oscillation of miR-17-5p was a conserved function between knee and hip AC. miR-140-3p was stably expressed in knee AC, though oscillated in SW1353 cell *in-vitro.* Thus, miRNAomes may be differentially regulated between man and mouse. Putative targets of the miRNAome were enriched for cartilage development, extracellular matrix, and inflammation ontologies. Therefore, the miRNAome emerges as a valuable effector of the clock mechanisms and future work will study the implications of daily fluctuations in miRNA abundance as on AC homeostasis.

Mona Saad: "Investigating the impact of elevated circulating mitochondrial DNA on melanoma metastasis and disease progression"

Melanoma is the leading cause of death from skin cancer, it tends to affect people of working age, having physical, psychological, social and financial implications. Better outcomes can be realised through new therapies or improved diagnostic and prognostic tool, developed from a better understanding of the biology of the disease.
Current investigation tools are invasive, costly and anxiety inducing. The use of blood tests to monitor melanoma remain unclear. My research work to date has focused on investigating the use and role of a novel non-invasive blood biomarker known as mitochondrial DNA (mtDNA) for melanoma screening and disease progression. MtDNA, a substance found circulating in the blood has been identified as a potential non-invasive tool in cancers.

As part of my research, I carried out a feasibility study under ethics from the Norwich research park (ETH2122-2327). Peripheral blood was obtained from 15-patients with biopsy proven metastatic melanoma prior to resection of the tumour and at 3 time points post surgery (4, 8 and 12 months). Cell-free mtDNA was obtained from these blood samples and quantified for 2 target mitochondrial genes (ND1 and CO2) using TaqMan PCR. This showed that circulating mtDNA levels were significantly higher in patients with known advanced melanoma compared to healthy patients. For most patients (13/15) the follow up levels of mtDNA were found to be lower compared to the levels detected at the time of surgery before the tumour was resected. Except for 2 patients, where there was an increase in levels after surgery. This appeared to occur in patients who developed a recurrence, requiring further treatment. Thus, mtDNA levels detected in the blood appeared to reflect the stage of melanoma.

The secondary aim of my research was to understand if circulating mtDNA in melanoma have an impact on metastatic disease progression. For this I investigated the role of mtDNA in melanoma progression, specifically looking at spread to the liver which is a known metastatic site. I found that a type of immune cell, Macrophages which would normally challenge cancerous cells exhibited pro-cancer features when exposed to mtDNA, which was extracted from the B16F10 cell line using a commercially available isolation kit.
The next natural progression step from this finding is to investigate if this behaviour in immune cells exposed to the mitochondrial DNA can be reversed through a novel agent that can be used in the treatment of aggressive advanced melanoma. Initial results thus far, suggest that circulating mtDNA detection has the potential to be a biomarker for melanoma disease progression. Moreover, melanoma-derived mtDNA has the potential to condition the pre-metastatic niche.

Kalina Popova: “Development of a microaerobic human intestinal organoid model to determine the role of the gut microbiome in Crohn’s disease”

Crohn’s disease (CD) is a chronic inflammatory bowel disease affecting approximately 120,000 people in the UK, characterized by symptoms such as diarrhoea, abdominal pain, tiredness, and weight loss. CD patients face increased risks of bowel obstruction, colorectal cancer, and often require surgery. Current treatments mainly provide symptomatic relief, and the precise causes of CD remain unknown. However, research indicates that environmental and genetic factors disrupt the homeostasis between the intestinal epithelium and resident microbiota, leading to reduced microbial diversity, gut epithelial leakiness, and an overactive mucosal immune response. Therefore, therapies aimed at altering the microbiota composition and restoring epithelial barrier function, represent a promising strategy in CD. However, research on microbiota-epithelium crosstalk is hindered by a deficiency of biologically relevant experimental models. My project aims to develop a physiologically relevant, microaerobic human intestinal organoid (HIO) model to study the role of the gut microbiome in CD.
The project builds on the microaerobic vertical diffusion chamber (VDC) system established in the Schüller lab, which maintains the growth and epithelial colonization of gut commensals under physiologically relevant hypoxia. The VDC system will be optimized to maintain a complex gut microbiome in the presence of HIOs for several days. The study will optimize the apical medium composition to establish a stable and diverse microbiome using stool-derived microbiota and HIOs from healthy donors (HDs) and CD patients, where HIOs exhibit donor specific genetic profiles, proving insights into disease modelling and personalised medicine. The optimization process will involve testing different media formulations and introducing continuous flow to prevent bacterial overgrowth and toxic metabolite accumulation.
The optimized microbiota-HIO VDC system will then be used to investigate microbiome-HIO crosstalk in CD and non-IBD controls. This “cross-over "study will determine how the epithelial origin (CD, healthy donors (HD) affect microbiome composition and metabolite production and how microbiome (CH, HD) influences epithelial barrier function and inflammatory response. HIOs from CD patients and HDs will be incubated with stool microbiota from CD patients and HDs. The influence on microbiome composition, metabolite production, epithelial barrier function, and inflammatory response will be evaluated via 16S rRNA sequencing, mass spectrometry, RNA-sequencing, TEER, dextran flux, immunofluorescence staining, RT-qPCR, and ELISA.

The project aims to establish a new human model system to better understand the role of the microbiota in CD, providing insights essential for developing therapies that address the root causes of CD and restore the balance between the microbiome and the epithelial barrier. The microbiota-HIO VDC model could also be applied in testing drug and probiotic candidates and can be adapted to other intestinal disorders associated with microbiome disturbances such as colorectal cancer. By using human-derived organoids, the model will be more physiologically relevant and personalised to CD than current mouse models, potentially reducing and replacing their use in research and preclinical studies.

Samson Balogun: "Passive Immunity to GP40 protects against Cryptosporidiosis"

Samson Balogun, Neil Hall, Mark Van Roosmalen, Geert Vertenten, Kevin Tyler

Institution Affiliation: Norwich Medical School, University of East Anglia; The Earlham Institute, MSD Animal Health.

Cryptosporidium spp. is a leading cause of diarrheal mortality, second only to rotavirus. In humans, it significantly contributes to diarrheal deaths, particularly in children under the age of five and immunocompromised individuals, accounting for approximately 200,000 fatalities annually. In the livestock industry, cryptosporidiosis results in substantial economic losses amounting to billions of USD per year. Despite its severe impact on human and animal health, there are currently no highly effective drug treatments or vaccines. Recently, MSD Animal Health introduced a newly licensed livestock vaccine based on passive transfer of immunity through bovine colostrum targeting the GP40 antigen of *Cryptosporidium parvum*. However, this vaccine has limitations, as it significantly reduces disease pathology but does not prevent parasite transmission or oocyst shedding into the environment.

This study aims to investigate the mode of action of passive immunity in colostrum by utilizing an *in vitro* cellular experimental model alongside microbiome profiling of vaccinated and unvaccinated calves. We demonstrated that a mouse monoclonal antibody (IgM) restricts the motility of *C. parvum* sporozoites in a live-cell immunofluorescence assay. To further characterize its binding specificity to extracellular antigens, we conducted a fixed-cell immunofluorescence assay, which confirmed the binding of the 4E9 antibody to the target antigen. This interaction significantly restricted parasite motility, paving the way for further functional studies using hyperimmune bovine colostrum in an *in vitro* cell model. We aim to assess how passive antibodies influence *Cryptosporidium* infection, parasite replication, and host cell responses, including pro-inflammatory cytokine production and host cell apoptosis. This research will provide proof of concept for the future development of a human vaccine against cryptosporidiosis.